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ORIGINAL ARTICLES

COMPARATIVE VALUES OF COMPLEMENT-FIXATION METHODS IN SYPHILIS*

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PRELIMINARY NOTE

IN 1898, Bordet discovered the hemolysins. Three years later, only nineteen years ago, he published the first account of complement fixation which later became known as the "Bordet-Gengou phenomenon." Speaking in terms of the side-chain theory of Ehrlich, the phenomenon of complement fixation depends upon the fact that complement, a substance present in greater or less quantities in the serum of all animals, will be bound or fixed, in the presence of an antigen and its specific antibody or amboceptor, a more or less firm union of the three resulting.

Bordet's first experiments were carried out using the following reagents: As antigen, a suspension of Bacillus pestis in normal sodium chloride solution, guinea pig's serum for complement. The two were placed in a test tube containing the serum from a horse which had been immunized against Bacillus pestis. The three were incubated for one hour, after which the hemolytic system consisting of the serum of a guinea pig which had been immunized against rabbit's erythrocytes, and a quantity of rabbit's erythrocytes were added. As the complement had been fixed in the first incubation, no hemolysis resulted.

Bordet's work was soon corroborated by Ehrlich and Morgenroth. Widal and Lesourd about this time made use of the reaction in the diagnosis of typhoid using bacillus typhosus as antigen. This was the first practical use made of the reaction.

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Although the work of Bordet and of Ehrlich coincided from an experimental standpoint, theoretically they held quite different views as to the underlying mechanism of the reaction of complement binding or fixation.

Bordet held and still holds that the antibody unites directly with the antigen and serves to sensitize it and prepare it for direct union with the complement as a mordant aids in the penetration of a dyestuff. Mordants are substances which are capable of attaching themselves to the substance to be dyed and subsequently to the dye itself. Substances of this kind are tannic acid (for basic dyes) and colloidal hydroxides (for acid dyes) such as the hydroxides of aluminium, tin, iron, and chromium. For example, the cloth to be dyed is treated with aluminium sulphate and acquires either by absorption or feeble combination, or both, a certain amount of the hydroxide; the fabric is then boiled in water with an acid dye (say Turkey red or alizarine which is a very slightly soluble acid, $C_{14}H_8O_4$) which unites with the hydroxide which has already united with the cloth. If we substitute the terms antigen for cloth, antibody for mordant, complement for dye, we can readily visualize in physico-chemical terms, Bordet's concept of the process of complement fixation.

Ehrlich, on the other hand, postulates the existence in cells of the body, of certain molecular groups which he calls receptors, which have the power to anchor or bind substances useful to them. Or the substance bound may be toxic to the cell and the cell may die or may be stimulated to the production of other receptors. These receptors, according to Weigert's law of overcompensation, ("overproduction theory"), are produced in excess and thrown into the blood stream. Ehrlich believes that these receptors or amboceptors play an all important rôle in complement fixation. According to him, the complement does not unite directly with the antigen but indirectly through the medium of the amboceptor which possesses a cytophile group which unites it with the antigen and a complementophile group which unites it with the complement.

In 1905, Wassermann and Bruck found that extracts of bacteria could be used in place of bacterial emulsions such as Bordet had used, also that extracts of tissue rich in bacteria might be used as antigen. Wassermann made use of the reaction in the diagnosis of tuberculosis, using as antigen lung tissue from tuberculous patients.

At this time the attention of the scientific world was drawn to syphilis, by the discovery of the Spirocheta pallidum by Schaudinn and Hoffman. In cooperation with Neisser, Wassermann and Bruck, May 10, 1906, made use of the reaction working with the serum from luetic monkeys using extracts of condylomata, luetic placenta, and so forth; as antigens. Just fourteen days later, Detre published his paper. He performed the reaction on six luetic and four normal persons, using tonsillar exudate, extract of leutic papules and pancreas as antigens. He obtained two positive reactions from the six leutic persons and all negative reactions from the normals.

Wassermann's first work on two hundred and seventy-five sera from luetic patients showed only nineteen per cent of positive reactions. This was due to the fact that elaborate controls and titrations now in use were lacking.

Following these pioneer papers many others appeared rapidly until the work done on serology has really assumed enormous proportions. At first the

Wassermann reaction was thought to be specific, that is, that each antigen produced its specific amboceptor in the blood stream of an immunized animal. However, the side-chain theory which Ehrlich elaborated to substantiate this view received a rude jolt, when in 1906 Citron showed that an extract from normal human red blood cells constitutes a fairly good antigen yielding positive reactions in about 80 per cent of luetic patients. In 1907, Weygandt obtained positive reactions on luetic sera using an extract of normal spleen as antigen. Müller and others used alcoholic extract of guinea pig heart the same year. These results directed attention to the fact that the antigen was probably a lipoid, and following this conception much work was done by many observers using sodium glycocholate and taurocholate, cholesterol, lecithin, vaselin, and so forth as antigens.

The investigators were so numerous, the technic employed so different, the results so antagonistic and confusing, that the reaction fell into disrepute, its opponents claiming that it yielded positive reactions in tuberculosis leprosy, scarlatina, in fact every infection besetting the human race. The painstaking efforts of honest workers, however, have shown pretty conclusively that we get little or no cross fixation in any diseases with the possible exception of frambesia (yaws) and leprosy. To this feature we will return later when we speak of our own experience with tuberculosis and other infections.

Coming now to our own work, it is our intention to outline the clinical and laboratory conditions existing in our institution calling attention to certain features of the work which we think might render our report valuable to other workers. Although the number of sera examined to date is not so great as from some of the larger laboratories of the country, we think that our results are fairly conclusive because of the following conditions existing.

(1) We have very close cooperation between the laboratory workers and the clinicians, the detail of this paper could never have been worked out without the hearty cooperation of the clinical staff which involved much extra work on their part, such as frequent consultation, repeated physical examination, and clinical observation.

(2) We have the patients under observation for a considerable time, enabling us to arrive at more accurate clinical diagnoses than we could ever hope to do otherwise. In every doubtful case several blood examinations were made at different times.

(3) The sera to be examined are all drawn on the day to be examined, by the same persons, using as nearly as possible the same technic each time. Nearly all sera examined from our hospital are examined within the first twenty-four hours after being drawn.

Early in our work our laboratory procedure consisted in subjecting each serum examined to the so-called classical Wassermann, together with the Gradwohl[1] modification of the Hecht test, using an alcoholic extract of beef heart as antigen. We soon discovered, however, that these procedures at best yielded a rather low percentage of positive reactions in patients in whom we had no difficulty in finding the various clinical pictures of lues affecting the central nervous system. This was due to the facts that the patients were suffering from late

lues, also nearly all of them had been subjected to vigorous treatment before being admitted to Longview.

After reviewing the various methods of complement fixation in lues, we decided to subject all sera to the following tests for the purpose of ascertaining which would be of the most value to us in our work.

(1) The Wassermann reaction, employing complement fixation in the water-bath at 37.5° C., using two antigens, one a plain alcoholic extract of beef heart properly titrated, the other the same extract reinforced with cholesterol, also properly titrated.

(2) The same technic as in (1) with the exception that complement fixation was carried out in the ice box at 2° C. for a period of ten hours, observing certain precautions to be alluded to later.

(3) The Gradwohl modification of the Hecht test with some slight alterations to be described.

If we investigate the results of other workers with the classical Wassermann with complement fixation in the water-bath, we find that the best figures give positive reactions in from 80 to 90 per cent of primary lues. In secondary lues, Kolmer reports 100 per cent, Boas 100 per cent, Craig 96 per cent positive reactions in the untreated cases. In untreated tertiary lues the reaction yields from 90 to 100 per cent positive results, while in the treated cases of tertiary lues the percentage of positive reactions ranges from 65 to 75 per cent.

Turning now to the results of the classical Wassermann with complement fixation in the ice box, together with the use of cholesterolized antigen we find rather conflicting reports. So far as the writer can learn, Jacobstahl[2] (1910) was the first to employ this method, fixing the complement in the ice box at 4° C. for a period of one and one-half hours. McNeil[3] was the first American to employ the method.

Concerning cholesterolized antigen Owen and Martin[4] state, "the published figures of positive reactions (with cholesterolized antigen) run as high as 10 to 20 per cent in patients who do not have lues." This most decidedly does not coincide with our experience. We have never, except in one instance, to be discussed later, obtained a positive reaction using the cholesterolized antigen on any patient on whom the diagnosis of lues could not be made either from the clinical history, the physical findings, or both.

Ottenberg,[5] Smith and McNeil[6] state that the cholesterolized antigen cannot be used with safety in the Wassermann with complement fixation in the ice box. Our experience will not enable us to concur in this conclusion.

THE HECHT-GRADWOHL TEST

The principle upon which this reaction works, that is, making use of the native complement and hemolysins in the blood serum, was first suggested by Tschernogouboff who employed guinea pigs' erythrocytes in the performance of the reaction, neglecting, however, the quantitative relationships of the test. Hecht also made use of the test using sheep's erythrocytes. His technic, however, lay open to the same objections as that of Tschernogouboff. It remained for Gradwohl to place the test on a reliable basis.

We employ practically the same method as outlined by Gradwohl* with the exception that we use fewer tubes (five to be exact) in the determination of the hemolytic index; we use three units, two units, and one unit of antigen in the test tubes of the back row, also we add an amount of red cells in the last incubation equivalent to one-half the amount of the hemolytic index. These alterations were suggested by Kolmer of Philadelphia. We have, furthermore, employed three types of antigen in our work, (1) the acetone insoluble as originally used by Gradwohl, also (2) a plain alcoholic extract of beef heart, and (3) a cholesterolized extract. The acetone insoluble is the most satisfactory. It is, however, more difficult of preparation. (For proof of this see tables that follow.)

With his technic Gradwohl has obtained fifteen per cent more positive reactions than with the classical Wassermann test. In his earlier paper he regards this as the best check on the Wassermann reaction of Neisser, Bruck, and Wassermann. In a later paper he concludes that the Hecht-Gradwohl test is not only a check, but, if positive, is diagnostic of lues. He states that he has never obtained a positive Wassermann reaction with a negative Hecht-Gradwohl test on the same serum, also that a doubtful Wassermann test should be thrown out in the presence of a negative Hecht-Gradwohl reaction on the same serum. He finds that the Hecht-Gradwohl test can be performed on 98 per cent of sera examined, his average hemolytic index is .5 c.c.

In the main our results coincide with those of Gradwohl. However, we find that there are some sera which may show complete inhibition in the first two tubes containing antigen, with only a partial inhibition in the third, or only the first tube may show complete inhibition with partial or no inhibiton in the last two antigen containing tubes, or all tubes containing antigen may show a partial inhibition, the control being clear. In this we are in agreement with A. J. Blaivas.[7] We may say here that those sera showing partial inhibition, with very few exceptions, were found by extensive clinical observation to have come from luetic patients. Furthermore, it is our opinion either with the Wassermann or Hecht-Gradwohl technic, if the technic has been accurate, sera showing partial inhibition should always be regarded with suspicion and it has been our experience that such sera were found on clinical observation to have come from patients with lues in one form or another. It is our custom to subject such sera to repeated examinations as it has been shown that the amount of antiluetic amboceptor in a patient's serum may vary from day to day in the same patient.

It has been our experience that the positive Wassermann reaction will give way to a negative Wassermann reaction under treatment, while the Hecht-Gradwohl test may remain strongly positive for some time afterward.

The reasons advanced as to why the Hecht-Gradwohl test is more sensitive than the Wassermann reaction are:

(1) There are two antiluetic amboceptors in serum from luetic patients, one is destroyed by inactivation, or heating to 56° C. as in the Wassermann test. As the serum is not inactivated in the Hecht-Gradwohl test this difficulty is obviated.

(2) By making use of the native complement and antisheep amboceptor in the patient's serum we avoid the excess of antisheep amboceptor over the com-

*For the details of the technic see Gradwohl's original paper, Jour. Am. Med. Assn., 1914, p. 240.

plement which may be so great as to throw a positive reaction to the negative side when the hemolytic system is added in the final step of the Wassermann reaction.

Concerning our Wassermann technic, without going into great detail we would like to emphasize a few points.

(1) Sera should be examined within the first twenty-four hours after being drawn.

(2) Complement should not be drawn from the guinea pig until needed for titration. It is the custom in our laboratory never to use complement after it has been drawn twelve hours.

(3) The concentration of cholesterin in the antigen should never exceed 0.4 per cent.

Regarding the Wassermann test with complement fixation in the ice box, we believe that the period of fixation should not exceed ten hours, neither should the temperature exceed 2° C. We use this procedure as it is the optimum time and temperature as worked out by Ruediger[8].

The technic is the same as in the classical Wassermann test up to the point of complement fixation when the tubes are placed in the ice box for ten hours at 2° C. At the end of this time we again wash the cells if the supernatant fluid shows any tinge of red. We also titrate the complement and amboceptor once again to make sure that they have not changed in titer. Some workers have said that the reason the ice box method yields more positive reactions is that the complement deteriorated in the time allotted to this method. This is not true in the light of our work.

After the tubes have been removed from the ice box it is well to warm them

TABLE I

Comparing (1) the Wassermann Reaction with complement fixation in the water-bath at 37.5° C.

(2) the Hecht-Gradwohl test, using the plain alcoholic extract of beef heart as antigen in 104 cases of lues involving the central nervous system.

Results (1)

The Wassermann test, complement fixation in the water-bath at 37.5° C.

	Number	Percentage
Positive	51	49.03
Borderline	7	6.73
Negative	46	44.23

(2)

The Hecht-Gradwohl test with plain antigen. Total number possible, 94.

	Number	Percentage
Positive	56	59.57
Borderline	5	5.21
Negative	33	35.1
Impossible	7	

	Number
Sera negative to the Wassermann test, but positive to the Hecht-Gradwohl test	9
Sera doubtful to the Wassermann test, but positive to the Hecht-Gradwohl test	6
Sera positive to the Wassermann test, but impossible to the Hecht-Gradwohl test	7

in the water-bath before adding the hemolytic system, as also suggested by Ruediger (loc. cit.).

The hemolytic system is added and the tubes returned to the water-bath at 37.5° C. for thirty minutes and read as before. We usually do two readings, one at the end of the last incubation, and one when all the cells in the tubes showing inhibition have settled out. The final reading is the one used for permanent record. We do not, however, consider a doubtful reaction with the ice box method as diagnostic of lues, but we do consider a reaction of the four-plus

TABLE II

Comparing (1) The Wassermann reaction with complement fixation in the water-bath at 37.5° C.
(2) the Hecht-Gradwohl reaction.
(a) Plain antigen and (b) plain antigen reinforced with cholesterol was used in both tests in 60 cases of lues involving the central nervous system.

Results (1)

The Wassermann test, using plain antigen, complement fixation in the water-bath at 37.5° C.

	Number	Percentage
Positive	22	36.66
Borderline	4	6.66
Negative	34	56.66

(1′)

The Wassermann test, using cholesterolized antigen, complement fixation in the water-bath at 37.5° C.

	Number	Percentage
Positive	25	41.66
Borderline	13	21.66
Negative	22	36.66

(2)

The Hecht-Gradwohl test, using plain antigen. Total number of cases possible, 55.

	Number	Percentage
Positive	28	50.9
Borderline	3	5.45
Impossible	5	
Negative	24	43.62

(2′)

The Hecht-Gradwohl test, using cholesterolized antigen in the same cases.

	Number	Percentage
Positive	37	67.2
Borderline	3	5.4
Impossible	5	
Negative	15	18.4

	Number
Sera negative to the Wassermann test, but positive to the Hecht-Gradwohl test with plain antigen	7
Sera doubtful to the Wassermann test, but positive to the Hecht-Gradwohl test with plain antigen	5
Sera negative to the Wassermann test, but positive to the Hecht-Gradwohl test with cholesterolized antigen	5
Sera doubtful to the Wassermann test, but positive to the Hecht-Gradwohl test with cholesterolized antigen	11
Sera positive to the Wassermann test, but impossible to the Hecht-Gradwohl test with both plain and cholesterolized antigen	4

variety as diagnostic of lues. We have never (with one exception) obtained such a reaction in a person who was not a luetic, but we have obtained three doubtful reactions with serum from patients in whom the diagnosis of lues could not be made.

Following are some tables compiled from data derived from examinations of over one thousand sera from patients admitted to Longview Hospital, together with four hundred and ten serum examinations on patients undergoing treatment for lues at the State Reformatory for Women, at Marysville, Ohio. Luetic cases only are considered in the accompanying tables. The remainder of the sera were from normal persons, that is, normal so far as could be determined by serologic and clinical examination.

The total number of sera from which Table I is made is 901. Only luetic cases are included in the accompanying tables.

The low percentage of positive reactions obtained in this as well as in our other series is due to the fact that nearly 100 per cent of all cases admitted to Longview Hospital have been subjected to long and vigorous treatment before the tests were performed. This fact accounts also for the considerable number of borderline or so-called doubtful reactions as serologic tests are less liable to be clear-cut positives or negatives in treated cases of lues than in untreated ones.

In Table II the percentage of positive reactions yielded by the Hecht-Gradwohl test over the Wassermann test is 10. The Hecht-Gradwohl test helped to a diagnosis of lues in 15 cases out of 104 where the Wassermann test was doubtful or negative. On the other hand, the Wassermann test helped to a diagnosis in 7 cases of lues in which the Hecht-Gradwohl test was impossible because of the absence of a hemolytic index.

TABLE III

Comparing (1) The Wassermann test with complement fixation in the water-bath for thirty minutes at 37.5° C.

(2) The Wassermann test with complement fixation in the ice box for ten hours at 2° C.

(3) The Hecht-Gradwohl test

(a) Plain alcoholic extract of beef heart and (b) the same extract reinforced with cholesterol constituted the antigens used in each test. Sera from 62 patients suffering with neurosyphilis in one form or another were subjected to the above tests. Sera from 319 nonluetic persons examined in the same series showed uniformly negative reactions to all tests.

Results (1)

The Wassermann test with complement fixation at 37.5° C. for thirty minutes in the water-bath with plain antigen in 62 cases of lues.

	Number	Percentage
Positive	17	27.41
Borderline	8	12.90
Negative	37	59.67

(1')

The Wassermann test with complement fixation at 37.5° C. with cholesterolized antigen in 62 cases of lues.

	Number	Percentage
Positive	23	37.09
Borderline	12	19.35
Negative	27	43.55

(3)

The Hecht-Gradwohl test with plain antigen. Total number of cases possible, 55.

	Number	Percentage
Positive	25	45.45
Borderline	8	14.54
Impossible	7	
Negative	22	40.00

(3′)

The Hecht-Gradwohl test with cholesterolized antigen in same cases.

	Number	Percentage
Positive	32	58.18
Borderline	4	7.27
Impossible	7	
Negative	19	34.54

	Number
Sera negative to the water-bath Wassermann test using the plain antigen but positive to the Hecht-Gradwohl test using the plain antigen	5
Sera doubtful to the water-bath Wassermann test using plain antigen but positive to the Hecht-Gradwohl test using plain antigen	5
Sera negative to the water-bath Wassermann test but positive to the Hecht-Gradwohl test, cholesterolized antigen being used in both tests	4
Sera doubtful to the water-bath Wassermann test, using cholesterolized antigen but positive to the Hecht-Gradwohl test using the same antigen	10

(2)

The Wassermann test with complement fixation in the ice box at 2° C. for ten hours with plain antigen in 62 cases of lues.

	Number	Percentage
Positive	53	85.48
Borderline	5	8.06
Negative	4	6.45

(2′)

The Wassermann test with complement fixation in the ice box at 2° C. for ten hours with cholesterolized antigen in 62 cases of lues.

	Number	Percentage
Positive	61	98.38
Borderline	0	
Negative	1	1.61

Twenty sera reacted negatively using two antigens in the Hecht-Gradwohl test, which yielded positive reactions to the Wassermann test, employing complement fixation in the ice box at 2° C. for a period of ten hours. Seven sera had no hemolytic index, hence the Hecht-Gradwohl test was not done. However, all of these sera showed a positive reaction to the Wassermann test employing complement fixation in the ice box.

In this series the Hecht-Gradwohl test, using the plain antigen, helped to a diagnosis of lues in twelve cases where the Wassermann test was either doubtful or negative. The Hecht-Gradwohl test, using cholesterolized antigen, helped to a diagnosis of lues in sixteen cases where the Wassermann test was negative. On the other hand, the Wassermann test made a positive diagnosis in four cases where the Hecht-Gradwohl test was impossible.

The interesting point observed in this series is the high percentage of positive reactions obtained with the Wassermann test when complement fixation in the ice box is employed. Under these conditions the Wassermann reaction yielded positive reactions in twenty cases where the Hecht-Gradwohl test was

negative, and a positive reaction in seven cases where the Hecht-Gradwohl was impossible. In other words twenty-seven cases of lues out of the sixty-two in this series would have been undiagnosed serologically if reliance had been placed upon one test alone.

We must bear in mind, however, that this does not mean that the Hecht-Gradwohl test would not have been positive had complement fixation been carried out in the ice box. We are at present working on this phase of the work and our results so far show that if complement fixation in the Hecht-Gradwohl test is carried out in the ice box at 2° C. for ten hours the Wassermann and Hecht-Gradwohl tests agree in practically 100 per cent of all cases. This feature will be dealt with in a future study.

TABLE IV

Comparing (1) The Wassermann reaction with complement fixation at 37.5° C. for 30 minutes.
(2) The Wassermann reaction with complement fixation at 2° C. for 10 hours.
(3) The Hecht-Gradwohl test
(a) Plain antigen and (b) cholesterolized antigen used in all tests in 20 cases of latent lues.

Results (1)

The water-bath Wassermann test using plain antigen.

	Number	Percentage
Positive	2	10
Borderline	1	5
Anticomplementary	0	
Negative	17	85

(1′)

The water-bath Wassermann test using cholesterolized antigen.

	Number	Percentage
Positive	2	10
Borderline	1	5
Anticomplementary	0	
Negative	17	85

(3)

The Hecht-Gradwohl test, plain antigen, 18 *cases possible.*

	Number	Percentage
Positive	5	27.77
Borderline	1	5.55
Impossible	2	
Negative	12	66.66

(3′)

The Hecht-Gradwohl test, cholesterolized antigen, 18 *cases.*

	Number	Percentage
Positive	9	50
Borderline	0	
Impossible	2	
Negative	9	50

(2)

The ice-box Wassermann test, plain antigen, 19 *cases possible.*

	Number	Percentage
Positive	15	78.95
Borderline	1	5.26
Anticomplementary	1	
Negative	3	15.79

(2')

The ice-box Wassermann test, cholesterolized antigen, 19 cases.

	Number	Percentage
Positive	17	89.47
Borderline	1	5.26
Anticomplementary	1	
Negative	1	5.26

In many of these cases the diagnosis of lues was unsuspected but being led by the positive serology, the diagnosis was later established by history and close physical examination.

TABLE V

Comparing (1) The water-bath Wassermann test, using plain antigen in 145 cases of lues under treatment.

(2) The Hecht-Gradwohl test, using plain antigen in 126 possible cases of lues under treatment.

Results (1)

The water-bath Wassermann test in 145 cases of treated lues.

	Number	Percentage
Positive	9	6.20
Borderline	3	2.06
Negative	133	91.72

(2)

The Hecht-Gradwohl, plain antigen, 126 cases possible, treated lues.

	Number	Percentage
Positive	26	20.63
Borderline	4	3.17
Impossible	19	
Negative	96	76.19

The Hecht-Gradwohl test yielded 14 per cent more positive reactions in this series of treated cases than did the Wassermann test.

TABLE VI

Comparing (1) The Wassermann test, complement fixation in the water-bath at 37.5° C. for 30 minutes.

(2) The Wassermann test, complement fixation in the ice box at 2° C. for 10 hours.

(3) The Hecht-Gradwohl test.

(a) Plain antigen and (b) cholesterolized antigen used in all tests of 157 cases of lues under treatment.

Results (1)

The water-bath Wassermann test using plain antigen in 157 cases.

	Number	Percentage
Positive	12	7.64
Borderline	5	3.18
Negative	140	89.17

(1')

The water-bath Wassermann test using cholesterolized antigen.

	Number	Percentage
Positive	17	10.82
Borderline	15	9.55
Negative	125	79.61

(3)
The Hecht-Gradwohl test using plain antigen, 128 *cases possible.*

	Number	Percentage
Positive	25	19.53
Borderline	3	2.34
Impossible	29	
Negative	100	78.12

(3′)
The Hecht-Gradwohl test using cholesterolized antigen in 128 *cases.*

	Number	Percentage
Positive	36	28.12
Borderline	14	10.93
Impossible	29	
Negative	78	60.93

(2)
The ice-box Wassermann test, plain antigen in 157 *cases.*

	Number	Percentage
Positive	69	43.94
Borderline	18	11.46
Negative	70	44.58

(2′)
The ice-box Wassermann test, cholesterolized antigen in 157 *cases.*

	Number	Percentage
Positive	83	52.86
Borderline	17	10.82
Negative	57	36.30

The data in Table VII serves to show that even using the acetone-insoluble antigen in the Hecht-Gradwohl test does not yield as high a percentage of positive reactions as does the Wassermann reaction employing complement fixation in the ice-box. However, this does not in any way militate against the earlier conclusions of Gradwohl, neither does it detract in the slightest degree from the efficiency of his test. We may repeat here that when the two tests are carried out with complement fixation in the ice chest in both, the results agree in about 100 per cent of the cases.

Our tables serve to show that methods of complement fixation in the ice chest are superior to methods of complement fixation in the water-bath. Why is this so? We may begin our answer to this question by stating that when a serologist uses only one method of complement fixation in lues he neglects the quantitative relationship of the Wassermann reaction. It is true that in the untreated secondary cases, or in cases of lues reacting strongly to the germ of syphilis, the original Wassermann technic will yield positive reactions in about 100 per cent, even though the weakest antigen, such as the plain alcoholic extract of beef heart, is used. In latent lues, treated lues and lues involving the nervous system such a procedure will yield at best a low percentage of positive reactions, whereas the more delicate methods will yield a much higher percentage of positive reactions. It is just as important, and infinitely more difficult, to establish a diagnosis in the latent types of lues as in the frank types, hence the need of more delicate reactions such as the Hecht-Gradwohl test and ice box fixation tests.

TABLE VII

Comparing (1) The water-bath Wassermann test, using plain and cholesterolized antigen.

(2) The ice-box Wassermann test, using plain and cholesterolized antigen.

(3) The Hecht-Weinberg-Gradwohl test, using the cholesterolized and the acetone insoluble antigen.

Of three hundred sera thus examined twenty-two were from patients suffering with neurosyphilis, seventy-seven were from cases of lues under treatment, the remainder were negative both clinically and serologically.

Results in twenty-two cases of neurosyphilis.

(1)

The water-bath Wassermann test using plain antigen.

	Number	Percentage
Positive	13	59.09
Borderline	2	9.09
Negative	7	31.81

(1′)

The water-bath Wassermann test using cholesterolized antigen.

	Number	Percentage
Positive	16	72.72
Borderline	2	9.09
Negative	4	18.18

(2)

The ice-box Wassermann test using plain antigen.

	Number	Percentage
Positive	21	95.45
Borderline	1	4.54
Negative	0	

(2′)

The ice-box Wassermann test using cholesterolized antigen.

	Number	Percentage
Positive	22	100
Borderline	0	
Negative	0	

(3)

The Hecht-Gradwohl test, cholesterolized antigen in 21 possible cases.

	Number	Percentage
Positive	15	71.42
Borderline	3	14.28
Impossible	1	
Negative	3	14.28

(3′)

The Hecht-Gradwohl test, acetone-insoluble antigen in 21 possible cases.

	Number	Percentage
Positive	17	80.95
Borderline	3	14.28
Impossible	1	
Negative	1	4.76

Results in seventy-seven cases of treated lues.

(1)

The water-bath Wassermann test, plain antigen, 77 cases.

	Number	Percentage
Positive	1	1.29
Borderline	3	3.89
Negative	73	94.81

(1′)

The water-bath Wassermann test, cholesterolized antigen.

	Number	Percentage
Positive	7	9.09
Borderline	2	2.59
Negative	68	88.31

(2)

The ice-box Wassermann test, plain antigen, 77 cases.

	Number	Percentage
Positive	22	28.57
Borderline	10	12.98
Negative	45	58.44

(2′)

The ice-box Wassermann test, cholesterolized antigen.

	Number	Percentage
Positive	36	46.75
Borderline	5	6.49
Negative	36	46.75

(3)

The Hecht-Gradwohl test, cholesterolized antigen, 68 cases possible.

	Number	Percentage
Positive	6	8.82
Borderline	16	23.53
Impossible	9	
Negative	46	67.64

(3′)

The Hecht-Gradwohl test, acetone-insoluble antigen, 68 cases.

	Number	Percentage
Positive	16	23.53
Borderline	9	13.23
Impossible	9	
Negative	43	63.23

The main reason why the ice box method of fixation is superior to the water-bath method is that the former test will detect the antiluetic reacting substances in the blood stream in higher dilutions than will the latter. To prove this one has but to perform the following simple experiment.

The serum used in this experiment yielded the following results when subjected to our routine tests using .1 c.c. of serum:

	Results
Water-bath Wassermann test	
Plain antigen	++++
Cholesterolized antigen	++++
Ice box Wassermann test	
Plain antigen	++++
Cholesterolized antigen	++++
Hecht-Gradwohl test	
Plain antigen	Positive (a + b + c + d −)
Cholesterolized antigen	Positive (a + b + c + d −)
Acetone Insoluble antigen	Positive (a + b + c + d −)

It will be readily seen that this serum contains a high concentration of the reacting substances of lues.

This serum was diluted as indicated in the diagram in two sets of tubes and

the Wassermann reaction employing water-bath fixation was performed on one set of tubes; the ice chest method of fixation was performed using the other set. Plain and cholesterolized antigens were used. The results of the water-bath fixation are recorded in the upper portion of the tube in the diagram, the results of the ice chest fixation in the lower. It is readily seen that the ice box fixation method using cholesterolized antigen yields a ++++ reaction in a higher dilution than does either the plain antigen in the ice chest or both the antigens in

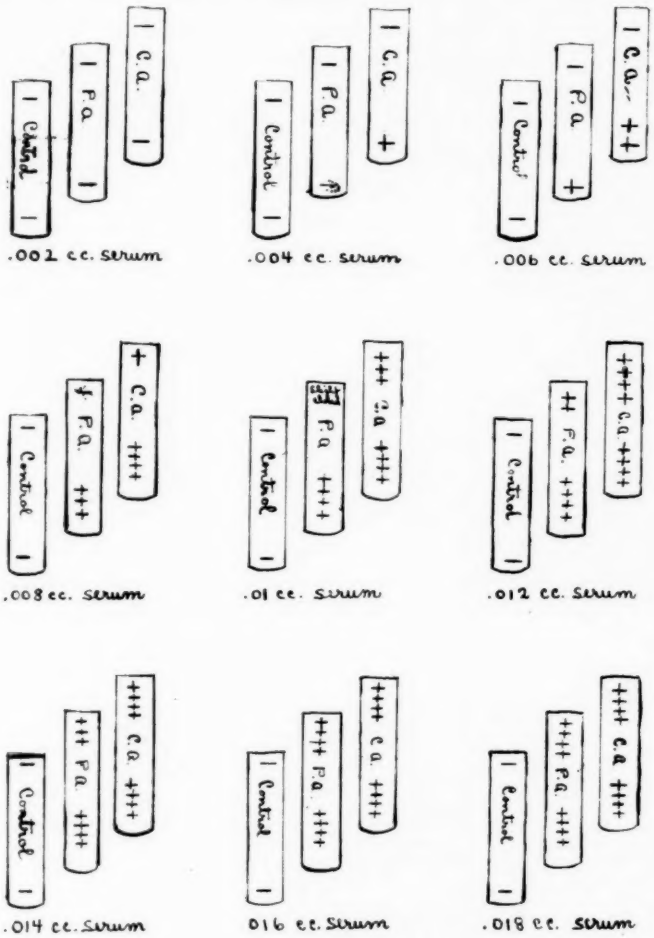

Fig. 1.

the water-bath. (Note results in the set of tubes containing .008 c.c. of patient's serum.)

One finds that when working with sera which react strongly to the original Wassermann test complement fixation takes place with great rapidity whereas with serums containing less of the reacting substances complement fixation takes place much more slowly. In this respect complement fixation follows the laws of adsorption, a point in favor of Bordet's theory concerning the phenomenon. This fact also points to an explanation of why the longer period of ice box fixation method using cholesterolized antigen yields a ++++ reaction in a higher

period of complement fixation in the water-bath. The main reason why long periods of fixation cannot be carried out in the water-bath is that complement deteriorates rapidly in the water-bath.

SUMMARY

1. Cholesterolized antigen properly prepared and titrated yields from 10 to 15 per cent more positive Wassermann reactions on luetic sera than does the plain antigen. We consider it a perfectly safe antigen to employ in the Wassermann reaction with complement fixation in the ice box at 2° C. for a period not longer than ten hours observing the precautions outlined in this paper. We have obtained but one positive reaction employing such methods in which the clinical findings, the history, or both, did not justify a diagnosis of lues. We may say here that we still have this patient under observation and there is a great possibility which may be later established that this patient has had lues.

2. The Hecht-Gradwohl test when positive in the temperate zone is diagnostic of lues. It will yield 15 per cent more positive reactions on luetic sera than does the classical Wassermann reaction. It may be employed in from 95 to 98 per cent of fresh sera (not over forty-eight hours old). It *does not* yield false positive results in tuberculosis.

The Wassermann test employing complement fixation in the ice box at 2° C. will yield a much higher percentage of positive reactions than does the Hecht-Gradwohl test employing complement fixation in the water-bath. With complement fixation under the same conditions, however, the tests practically agree.

The three serologic reactions appear in the serum and disappear under treatment in the following order:

The ice box Wassermann reaction is the first to appear positive, the Hecht-Gradwohl test follows, the water-bath Wassermann reaction appearing last. Under treatment the water-bath Wassermann reaction disappears first, the Hecht-Gradwohl reaction next, the ice box Wassermann reaction last.

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PRIMARY LYMPHOSARCOMA OF THE STOMACH. A REPORT OF TWELVE CASES

By A. C. Broders, M.D., and A. E. Mahle, M.D., Mayo Clinic, Rochester, Minnesota

PRIMARY lymphosarcoma of the stomach compared with carcinoma of the stomach occurs in the proportion of 1 to 68 according to the records of the Mayo Clinic. But few cases are reported. Ruppert, in 1912, collected twelve cases from the German literature, to which he added one. A few cases have appeared from time to time in American, English, and French literature.

Fig. 1.—(a190100). Lymphosarcoma of the stomach. The rolled-edge border gives it the appearance of a mushroom lying on the gastric mucosa.

Fig. 2.—(a190100). Section of lymphosarcoma shown in Figure 1. The neoplasm has pushed up the mucosa and invaded the musculature.

In our series of twelve cases of lymphosarcoma of the stomach observed at the Mayo Clinic from January 1, 1913, to December 1, 1920, the average duration of clinical symptoms was six and eight hundredths months. Eleven patients gave a history of loss of weight, nine of pain, seven of vomiting, and two of bleeding; two had histories suggestive of previous ulcer. The average age of the patients was forty-six and twenty-five hundredths years; the youngest patient was 16, and the oldest, 62. Eleven were males.

In seven cases the clinical diagnosis was carcinoma; in one ulcer; in one abdominal tumor, probably inflammatory; in one lesion of the stomach, probably malignant; in one pyloric obstruction; in one upper abdominal tumor, probably of the pancreas.

Resection was performed in six cases; in the other six the condition was found to be inoperable on exploration. The neoplasms were located for the most part in the pyloric portion of the stomach.

Fig. 3.—(a265434). Lymphosarcoma of the stomach associated with ulceration of the mucosa.

Fig 4.—(a265434). Section of lymphosarcoma shown in Figure 3. The neoplasm has invaded the musculature; in one place it has extended through to the serosa.

PATHOLOGY

Macroscopic Examination.—On section of the stomach the rolled edged border of the neoplasm with its raised surface presents the appearance of a mushroom, lying in the folds of the normal mucosa (Figs. 1 and 2). The surface of this may or may not be ulcerated; the ulceration may be shallow or deep (Figs. 3 and 4). On section the neoplasm is fairly soft and resilient and pale

straw colored; it is limited largely to the submucosa, but here and there invades the musculature. The thickness of the wall of the stomach, including the tumor, may be as much as 3 cm. The growth in one of the cases in this series was very extensive. It appeared to extend directly through the muscle and serosa into the gastrocolic omentum. The surrounding lymph nodes were also extensively involved.

Microscopic Examination.—The tumor-cells massed about the glands of the gastric mucosa involve the entire space of the submucosa and extend down

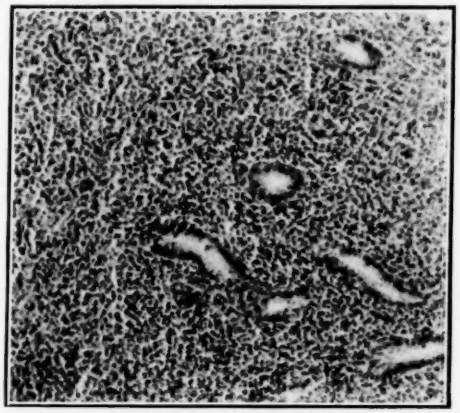

Fig. 5.—(a316187). Low power photomicrograph showing the neoplastic cells massed around the gastric glands.

Fig. 6.—(a336365). Low power photomicrograph showing the neoplastic cells lying between the muscle fibers.

between the muscle fibers (Figs. 5, 6, and 7). The cells resemble for the most part those of the germ center with areas of normal lymphocytes scattered here and there. These cells are larger than normal lymphocytes, usually irregular and often contain prominent single nucleoli (one-eyed cells). The tissue is recognizedly lymphoid, but the absence of germ centers and the fact that the entire section is but a homogeneous structureless mass of cells with the exception of a few lymphocytes are particularly striking. In two of the six cases

in which a portion of the stomach was resected the lymph nodes were involved, and in one the serosa. In four of the six cases which proved to be inoperable at the time of exploration the adjacent lymph nodes were involved (Fig. 8).

RESULTS

Two of the six patients who had resections died of peritonitis following operation. One patient died four months after operation with a recurrence in the lower bowel, liver, and remainder of the stomach. One returned prac-

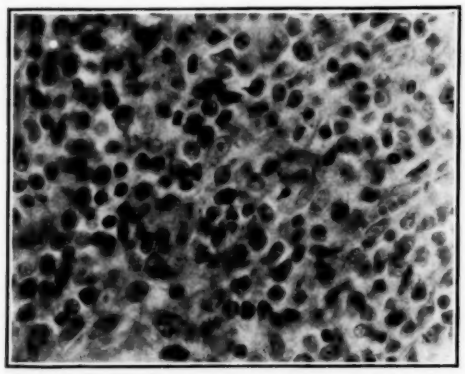

Fig. 7.—(a336365). High power photomicrograph of a neoplastic area shown in Figure 6.

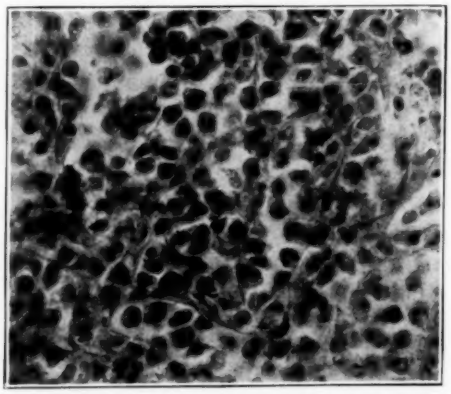

Fig. 8.—(a205800). High power photomicrograph of a lymphosarcomatous area of a lymph node lying close to the stomach.

tically five months after operation in poor condition; he had lost 20 pounds and he had a mass in the left epigastrium which probably was a recurrence. Another returned practically seven months after operation, apparently with a recurrence. The remaining patient was operated on too recently to be considered in the results. Four of the six patients who had explorations are dead. They lived for from six weeks to four months after exploration. Information has not been received from the remaining two patients.

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THE ETIOLOGY OF ACUTE INFLAMMATIONS OF THE NOSE, PHARYNX AND TONSILS*

By Stuart Mudd, M.D., Samuel B. Grant, M.D., and Alfred Goldman, M.D., St. Louis, Mo.

(*Continued from page* 190.)

VASOMOTOR REACTIONS TO CHILLING

TEMPERATURE changes in the skin and exposed mucous membranes were thus established as valid criteria, under the conditions of our experiments, of vasomotor tone. The graphs to follow actually show surface temperature changes; their significance is of alterations in blood supply. Obviously, fall in temperature means reflex vasoconstriction, a rise, vasodilation.

The vasomotor responses to chilling of a distant area of the body surface exhibited in the exposed skin of the forehead and of the mucous membrane of the soft palate and oropharynx, respiration and other conditions being carefully controlled as described above, may readily be made out from Fig. 4.

The two lower curves of this figure are composite graphs of four experiments of similar pattern. The points chosen for plotting are: the first and last readings of each experiment, the readings immediately before and after each change in experimental conditions, and the first point of maximum response of the mucous membrane to changed conditions; each point graphed is the average value of the corresponding point of the four experiments. Time is plotted on the horizontal axis, temperature on the vertical; the space between the ruled lines represents ten minutes on the abscissa, one-half degree centigrade on the ordinate. The character of the lines connecting the points gives the nature of the experimental conditions, as indicated in the legend.

While respiration is quiet and the subject wrapped, mucous membrane and skin temperatures remain constant. Deepening of respiration affects only inconsiderably the skin temperature, but causes a depression of mucous membrane temperature amounting to .68° C., which reaches its maximum after 25.4 minutes, and then, during the remaining 8.5 minutes before chilling begins, remains virtually constant.

The subject was chilled by unwrapping him and directing the draft of an electric fan against the lower thoracic region of his back. With the start of this process, a marked depression both of mucous membrane and of skin temperature begins. The maximum fall of mucous membrane temperature is 1.42° C., reached in 18.4 minutes. The synchronous point on the skin curve represents a drop of 1.73° C. The skin curve falls away a little more sharply

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The bibliography will appear at the end of the third section of this paper in the March number of the *Journal of Laboratory and Clinical Medicine*.

than the mucous membrane curve. However, even if the vasoconstriction in skin and mucous membrane were to follow an identical course, we should expect, on mechanical grounds, this difference in the curves; for the more exposed forehead would, of course, lose heat more rapidly than the mucosa of the palate and pharynx.

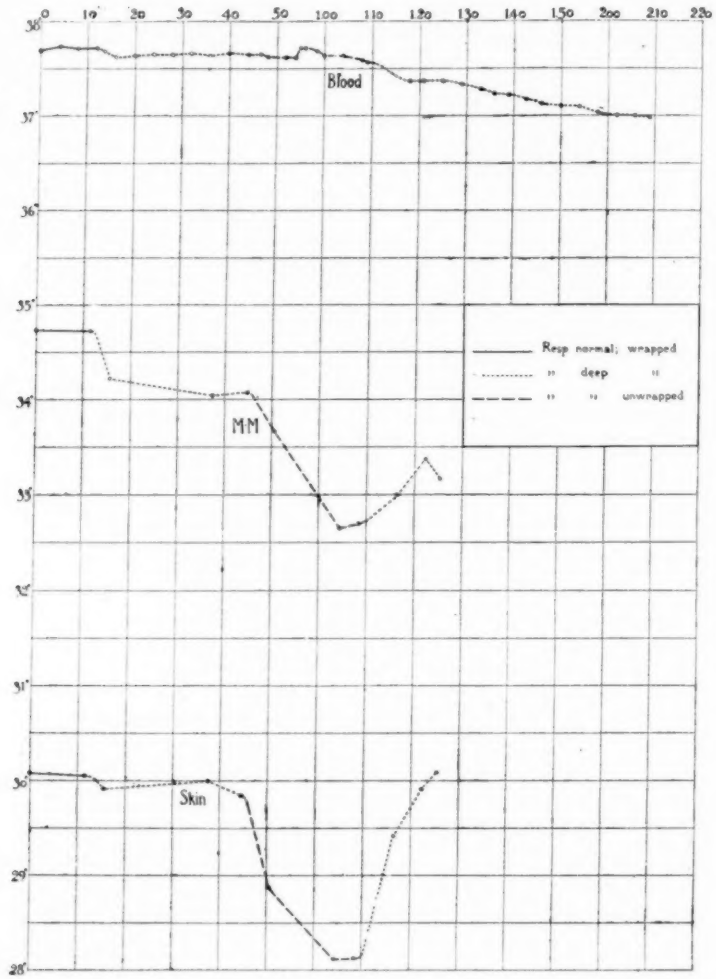


Fig. 4.—Effects of chilling body surface. Respiration controlled. Composite graphs of four experiments (temperatures of skin and mucous membranes of oropharynx and soft palate) and two experiments (blood temperature). Subject chilled by unwrapping, with draft of electric fan on back.

When it was seen the mucous membrane temperature had ceased to fall, the fan was turned off and the subject again wrapped. Here a disparity in the behavior of skin and mucous membrane vessels appears. The skin temperature climbs steeply and surmounts the level at which chilling began. The skin "reacts," as is commonly said. But the mucous membrane temperature rises only .73° C. Its maximum recovery is reached after 12.7 minutes and is .69° C. below the last point before chilling. During the remaining 3.5 minutes of observation, it falls .21° C.

No explanation in mere physics is to be found for the mucous membrane temperature remaining depressed; if its vessels returned to the same tone as before chilling, the temperature curve should return to control level. Yet this same incomplete recovery after chilling was noted in all the crucial experiments upon soft palate and pharynx, alike the early determinations with respiration uncontrolled and the final ones with respiration controlled. Measurement of the respiration records for the four experiments graphed in Fig. 4 was made, and the mean figure arrived at for respiratory amplitude before chilling differed from that after chilling by only a fraction of one per cent. In those experiments in which respiration was slightly deeper before as well as in those in which it was a few per cent deeper after chilling, the failure of the mucous membrane temperature to regain its former lever is usually evident. We are forced to conclude, therefore, that the vasoconstriction and ischemia reflexly produced in the palatine and pharyngeal mucous membrane by chilling the body surface persist in part for some time at least. The skin in our experiments has tended more completely to regain its blood supply. The tonsils have tended to regain their blood supply even more completely than the skin; in some instances actually becoming hyperemic on rewarming (Grant, Mudd and Goldman, 1920).

The uppermost curve in Fig. 4 is a composite of two blood-temperature control experiments which followed the same pattern as the two lower curves. For discussion see above.

A few experiments, illustrating typical reactions in the several regions of the nose and throat, may now be briefly considered:

EXPERIMENT A.—M.M. thermopile on anterior half of *soft palate*. Skin thermopile on forehead. Respiration, 18 per minute. Mouth open, nose breathing. Thoracic and abdominal pneumographs. Room temperature 17.9° to 18.8° C.; time, 3:55 to 5:16 P.M.

Experiment A from 0:00 to 0:50, shows the typical picture described for the composite graph above save that the anterior palatine mucous membrane is not affected by deepening respiration. At 0:50 the subject's feet were exposed, wrapped in cold wet towels, and the electric fan turned on them. This seems to have been without effect upon the forehead, but was followed apparently by a slight depression, .29° C., of mucous membrane temperature. This effect was not sufficiently definite to warrant much emphasis, but is at least suggestive in view of the possible efficacy of wet feet in exciting colds.

Administration of amyl nitrite at 1:03, while the feet were still being chilled, was followed by a steep rise in mucous membrane and skin temperatures with quick return to normal. The break in the skin curve is meant to indicate that it may have risen above 30.50° C. in the interval, 1:02 to 1:08 during which no skin readings were made.

The uppermost curve in Fig. 5 is a blood temperature control.

EXPERIMENT B.—M.M. thermopile on posterior wall of *oropharynx*. Skin thermopile on forehead. Respiration, 18 per minute. Nostrils plugged with cotton, mouth breathing. Thoracic and abdominal pneumographs. Room temperature, 19.0° to 20.6° C.; time, 10:11 P.M. to 12:33 A.M.

The curve of Experiment B (Fig. 6) shows the pharyngeal mucous membrane reacting in essentially the manner indicated in the composite graph, and shown also for the palatine membrane in Experiment A. The difficulties in technic are considerably greater with the thermopile applied to the pharynx than to the palate, however, and the curve is never so smooth. The effects of moving the pharyngeal wall against the thermopile terminals by coughing, swallowing or clearing the throat, is shown at 0:27, 0:50.5 and 1:41. Presum-

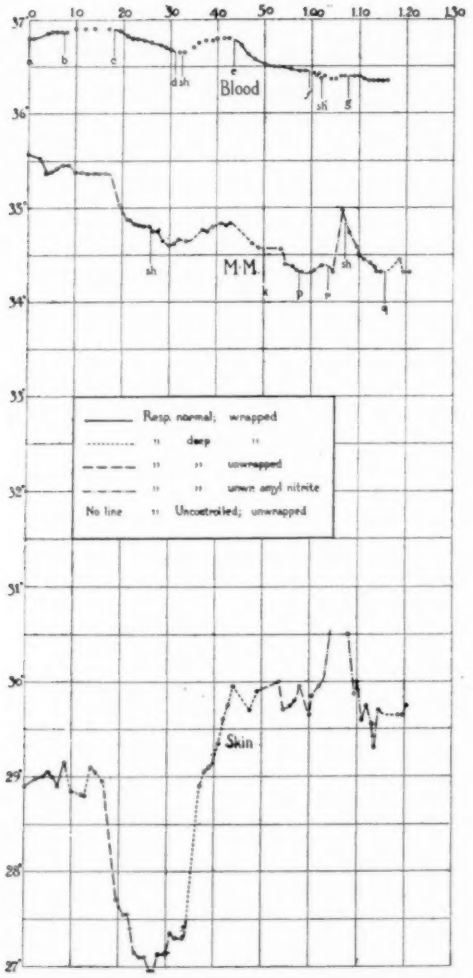


Fig. 5.—Chilling and amyl-nitrite effects. Experiment A (temperatures of skin and mucous membrane of soft palate) and Experiment K (blood temperature): *a*, wrapped; *b*, unwrapped; *c*, wrapped; *d*, unwrapped, fan on back; *sh*, shivering begins; *e*, fan off, wrapped; shivering stops; *f*, unwrapped, cold, wet towel to back; *sh*, is shivering; *g*, dried and wrapped; *k*, cold, wet towels to feet, fan on feet; *p*, more cold water poured on towels around feet; *r*, respiration is exaggerated; *q*, fan off, feet dried and wrapped.

ably two factors play a part in the sudden rise in temperature produced; the momentarily increased pressure between terminals and mucous membrane slightly increased, mechanically, the temperature of the former; the painful mechanical irritation of the mucosa by the metal terminals and applicator probably caused a transient blush. The rises in temperature, which took place

before chilling, although apparently elicited by less movement, were much more marked than that which occurred during the active vasoconstriction with chilling—1.77° and 2.69° as compared with .98° C.

The effect upon skin temperature of inhaling amyl nitrite is shown (2:17). A rise of .50° C. is again produced.

It is to be remembered that, since the mucous membrane and skin curves of each experiment are synchronous, the annotations, although drawn in as though applying only to one curve, apply equally to both.

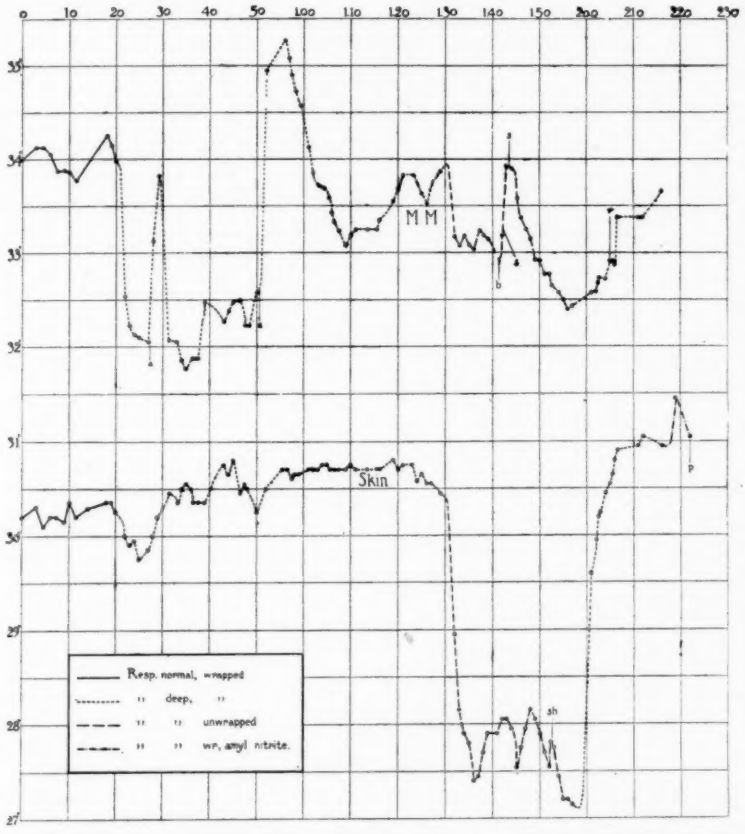


Fig. 6.—Effects of chilling, mechanical irritation and amyl nitrite. Experiment B (temperatures of skin and mucous membrane of oropharynx): *a*, coughs and clears the throat; *d*, coughs and clears throat; *b*, clears throat and swallows; *s*, swallows; *sh*, begins shivering; *r*, misses several respirations; *p*, exact time of point *p* not known.

EXPERIMENT C.—M.M. thermopile on posterior wall of *nasopharynx*. Skin applicator on forehead. At first, nostrils plugged, breathing through mouth; later, breathing through nose, mouth open. Respiration, 18 per minute. Thoracic and abdominal pneumographs. Room temperature, 15.7° to 16.9° C.; time, 4:15 to 5:45 P.M.

With the anterior nares plugged and the soft palate raised in mouth breathing, the nasal chamber was virtually a closed cavity, whose temperature did not vary with the rate of blood flow through its walls. Correspondingly, the mucous membrane curve (Fig. 7) showed no certain change when, at 0:08.5, respiration was deepened, nor at 0:22, when the subject was unwrapped

and chilled with the electric fan. The skin curve, on the other hand, dropped off characteristically, to recover when the subject was rewrapped.

At 0:46 the nostrils were unplugged and nose-breathing began. The nasopharynx at once came into free communication with the outer air, and was cooled by each respiration, and became dependent upon its blood supply for maintenance of its temperature. When equilibrium had been reached, the subject was again, at 1:07.5, unwrapped and chilled with the fan. Mucous membrane and skin temperatures fell together, the former reaching a maximum depression of 1.83° C. in six minutes, the latter of 1.95° in eight minutes. After

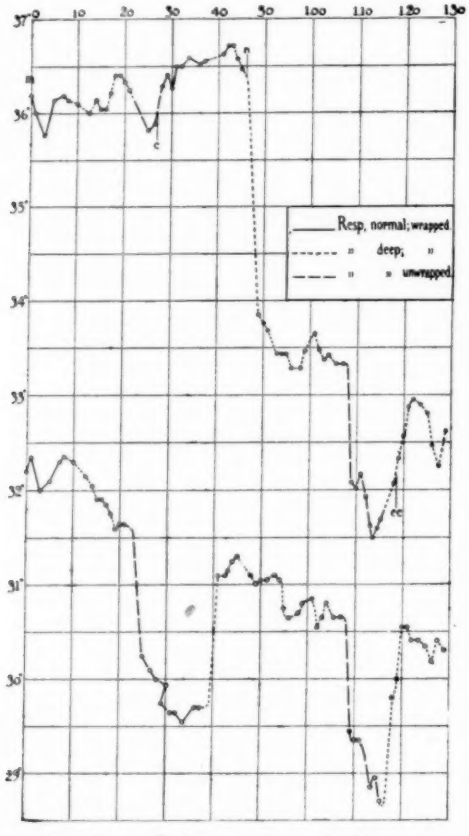


Fig. 7.—Chilling effect. Experiment C (temperatures of skin and mucous membranes of nasopharynx): *m*, nostrils plugged, breathing through mouth; *c*, coughs; *n*, plugs removed from nostrils, breathes through nose; *cc*, coughs three times.

rewrapping at 1:16, the mucous membrane temperature rose in six minutes to a maximum point .38° below the control level, then fell slightly; the skin temperature mounted in four minutes to a maximum only .10° below control level.

In the experiments of the first series a single exception to the rule that mucous membrane temperature, upon wrapping after chilling, does not regain its original level,was found in the experiment performed upon a faucial tonsil. In this case mucous membrane temperature rose well above control level. With this idea of determining whether or not this behavior was characteristic, and because of the peculiar importance of the tonsils as a site of infection, a second

series of experiments was performed with especial reference to the tonsillar reactions. These confirmed the earlier observation.

The curves on the right of Fig. 8 are a composite of four experiments upon tonsil and skin; on the left are shown for comparison composites of two experiments with the soft palate and one with the oropharynx as mucous membrane site of application.

In the tonsillar curves chilling begins at 0:15; mucous membrane and skin temperature fall away steeply together. Upon rewrapping at 0:27 skin temperature returns not quite to control level, tonsillar temperature considerably above it. In the palatine-pharyngeal curve, on the other hand, the character-

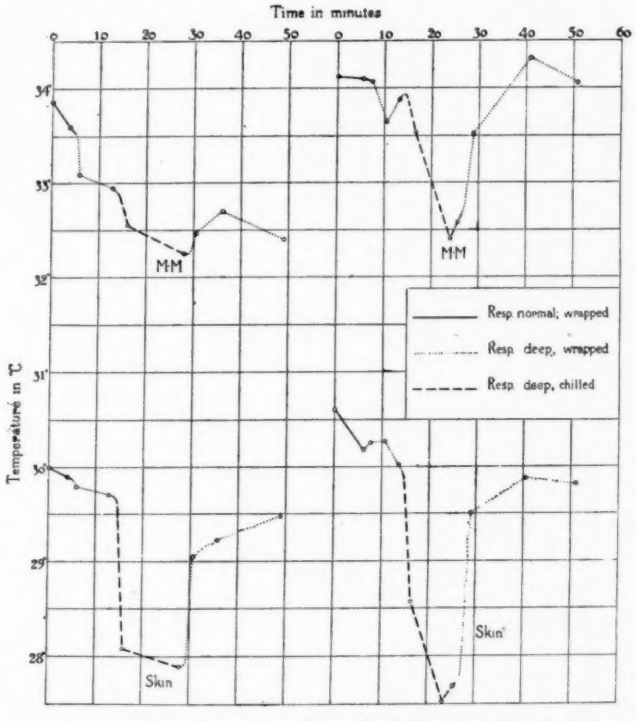


Fig. 8.—Reactions to chilling and to rewarming. On left, composite of three experiments. (Temperatures of skin and mucous membrane of soft palate and oropharynx.) On right, composite of four experiments. (Temperatures of skin and mucous membrane of faucial tonsils.)

istic failure to regain normal relaxation of vasomotor tone after chilling is again evinced.

EXPERIMENT D.—M.M. thermopile on left *faucial tonsil*. Skin application on forehead. Nostrils plugged, breathing through mouth. Respiration, 14 per minute. Thoracic and abdominal pneumographs. Room temperature 17.3° to 19.45° C.; time, 11 A.M. to 12 M.

Experiment D illustrates the same reactions as the composite. With the beginning of chilling at 0:12 skin and mucosa lose heat together, to regain it on rewrapping at 0:17.5. By 0:34.5 the mucous membrane temperature had risen to 33.85° C., 1.32° above the level at which chilling began. At 0:35 an ampule of amyl nitrite is inhaled. The vasodilation in the already hyperemic

tonsillar mucous membrane was evidently insufficient to counterbalance the effect of lowered general blood pressure, so that a fall in temperature resulted. The skin temperature on the other hand, rose characteristically with the amyl nitrite. As the flush passed, skin temperature fell and mucosal temperature rose to its former level.

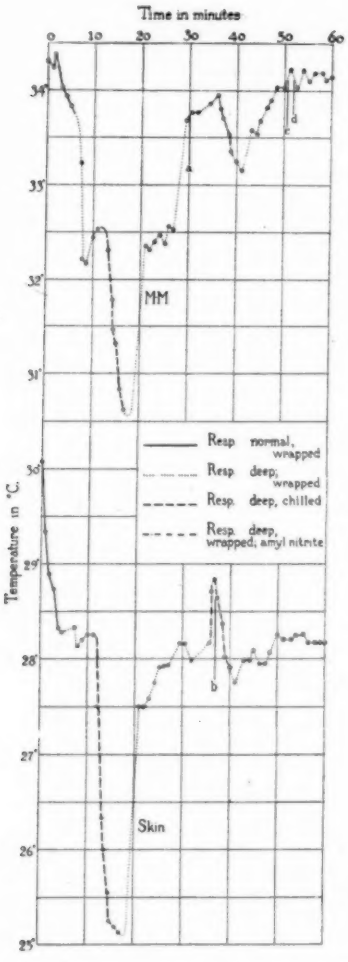


Fig. 9.—Chilling, recovery and amyl nitrite reactions. Experiment D (temperatures of skin and mucous membrane of faucial tonsil): *a*, applicator seen to be all right; *b*, flush fading; *c*, respiration shallow; *d*, breathes deeper.

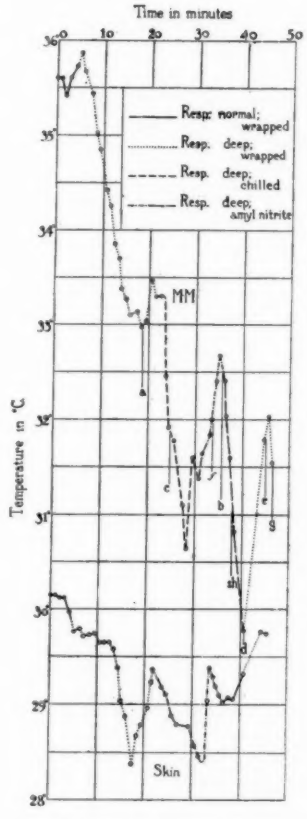


Fig. 10.—Chilling and amyl nitrite reactions. Experiment E (temperatures of skin and mucous membrane of faucial tonsil): *a*, clears throat; *c*, coughed; *f*, face flushed; *b*, flush passing, amyl nitrite taken away; *sh*, shivering; *d*, clears throat; *e*, respiration too shallow, deepened; *g*, subject fainted.

EXPERIMENT E.—M.M. thermopile on left *faucial tonsil*. Skin applicator on forehead. Nostrils plugged; mouth breathing. Respiration 14 per minute. Thoracic and abdominal pneumographs. Room temperature 17.5° to 18.55° C.; time, 10:56 to 11:43 A.M.

Fig. 10 with the beginning of chilling at 0:23 shows the usual temperature fall; the recovery curve was terminated prematurely by the subject unfortunately fainting. Amyl nitrite was administered during chilling at 0:32.5. Vaso-

dilation here evidently more than counteracted blood pressure fall, for the skin and mucous membrane temperatures each rose steeply.

The reactions of the *nasal cavity* have recently been found to be similar in quality to those of the oropharynx and nasopharynx, but quantitatively much more striking. Twelve experiments have been performed on seven different subjects of Aryan, Semitic and Mongolian stock. The sites tested are the nasal septum, inferior and middle turbinates, and inferior and middle meati, all, because of difficulty in application farther back, in the anterior half of the nasal cavity.

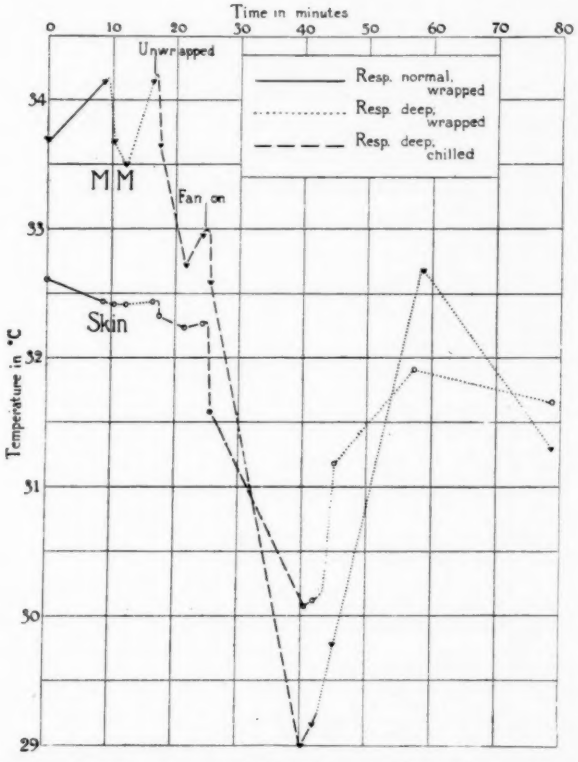


Fig. 11.—Reactions of nasal cavity to chilling and rewarming of body surface. Composite graph of seven experiments. (Temperatures of skin and of mucous membrane of nasal septum, inferior turbinates and middle meatus, in anterior half of nasal cavity.)

In every case chilling of the body surface has resulted in reflex vasoconstriction in the nasal mucous membrane, rewarming in vasodilation. Striking vasodilation occurs on inhaling amyl nitrite.

The reflex to the nasal mucous membrane, as is also the case for the mucous membranes of the palate, nasopharynx, and oropharynx, and for the skin of the base of the neck, appears to have a lower threshold than the corresponding reflex to the skin of the forehead. Merely unwrapping the subject in a room of 14.0° to 18.0° C. in a number of instances resulted in no change or a very slight fall in skin temperature and a fall in mucous membrane temperature of 1.5° to 2.0° C., or even more.

Application to the nasal mucosa of the thermopile terminals was extremely irritating and resulted in discharge of considerable amounts of clear mucus. The discharge was both on the side of the cavity in which application was made and on the opposite side, although more abundant on the former. It seemed to be little if at all affected by the marked shrinkage of the mucous membrane during chilling. In three experiments the subject thought secretion was slightly more copious during chilling; in one he thought it slightly less. In others no change in secretion was noted. These last observations bring out two facts:

(1) Discharge from the nasal cavity may be reflex and may occur in regions of the nose not directly irritated.

(2) Rhinorrhea is not necessarily accompanied by vasomotor turgescence in the nasal cavity.

The nasal vasomotor reactions are illustrated by Figs. 11 and 12.

Fig. 11 is a composite of seven experiments of similar pattern performed upon four subjects. The sites used were the right and left sides of the nasal septum, the right and left inferior turbinates, and the left middle meatus.

A transitory fall of 0.6° C. in mucous membrane temperature followed deepened respiration. On unwrapping the subject, mucous membrane temperature was depressed 1.4° C., skin temperature synchronously only 0.2° C. Turning an electric fan on the subject's lower back resulted in a further mucous membrane fall of 3.7° C. and a skin fall of 2.1° C. Maximum recovery for mucous membrane was 3.7° C. (71 per cent); for skin the corresponding recovery was 1.8° C. (77 per cent).

EXPERIMENT F.—M.M. thermopile on anterior end of *left inferior turbinate*. Skin applicator on forehead. Mouth closed; nasal breathing. Respiration 14 per minute. Thoracic and abdominal pneumographs. Room temperature 16.0° to 17.0° C.; time, 1:14 to 2:48 P.M.

Fig. 12 illustrates in an individual experiment the reactions brought out in the composite. With unwrapping at :12.0 the skin temperature is not depressed for two and a half minutes; the mucous membrane temperature in like time falls 1.6° C. The pronounced drop in both mucous membrane and skin curves with fan on is interrupted by a sharp rise following amyl nitrite administration at :23.25, amounting, in the case of the mucosa, to 3.9° C., in that of the skin to 1.1° C. After unwrapping, the mucous membrane temperature in this experiment slightly more than regained its level of before chilling,—(in most of the experiments it remained depressed). Inhalation of amyl nitrite in this flushed condition of the mucous membrane resulted in a momentary depression of 0.2° C., followed by a rise of 0.6° C. Skin temperature rose 1.0° C. approximately as before. The experiment ends with a profound vasoconstriction of mucous membrane and skin vessels incident on a second chilling with the fan.

Checking of quantitative results by *qualitative observations of redness:* The experimental analysis outlined above adequately proves, it seems to us, that chilling of the body surface reflexly produces vasoconstriction in the vessels supplying the normal mucous membranes of the palate, tonsils, oropharynx and nasopharynx, and of the nasal cavity. However, in order to secure still further corroboration, observations of the appearance of the mucous membranes and their blood vessels were made.

Twelve experiments were performed. Of the five observers who noted the appearance of the buccal and oropharyngeal membranes before and after direct exposure of those membranes to cold air, all were of the opinion that, with the direct chilling, blanching occurred. Of the six observers who noted the

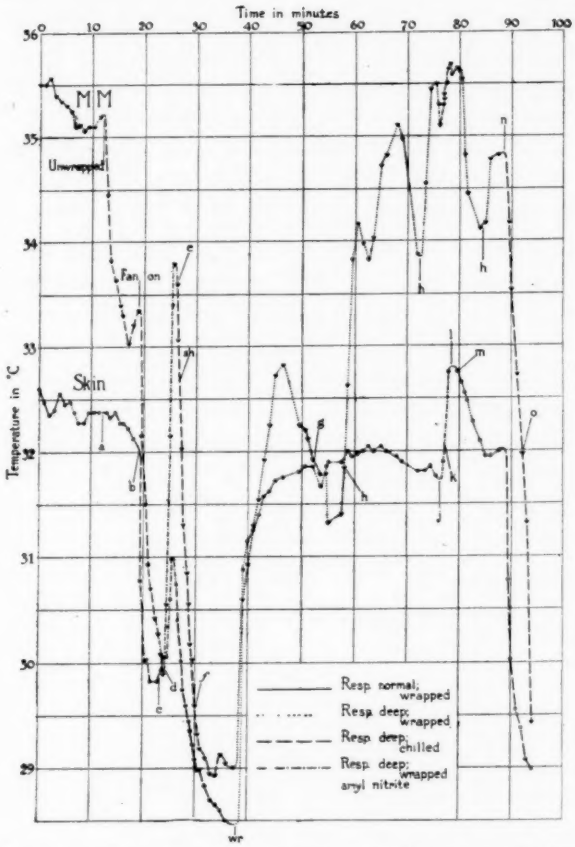


Fig. 12.—Chilling, rewarming and amyl nitrite reactions. Experiment F (temperatures of skin and mucous membrane of left inferior turbinate, anterior end): *a*, subject unwrapped; *b*, fan on; *c*, amyl nitrite inhalation begins; *d*, face has begun to flush; *e*, flush fading, stops inhaling; *sh*, shivers; *f*, is shivering; *wr*, fan off, warmly wrapped; *g*, *m. m.* applicator feels to subject to be in correct position; *h*, *m. m.* applicator readjusted so that subject can feel pressing against turbinate; *j*, amyl nitrite administered; *k*, face flushed; *l*, stops inhaling amyl nitrite; *m*, flush fading; *n*, unwrapped, fan; *o*, is shivering hard.

appearance of the soft palate and the seven who studied the normal oropharynx before and after chilling of the body surface, all said that the membranes paled while the skin was being chilled. Only one observer specifically studied the reaction of the tonsils. He said that they blanched during the skin's chilling. Of the three observers who watched the changes following rewrapping, one simply said that reddening occurred; two said that reddening occurred only to a slight extent.

The first, i. e., the response of the mucous membranes to direct chilling, was not suitable to thermogalvanometric study. The observation that blanching occurred is, however, in harmony with observations upon direct cooling of the nasal mucous membrane made by Cocks (1915). The other points observed are wholly in accord with the quantitative studies.

Results in harmony with our own have been published by Tschalussow (1913) who used the nose with posterior nares packed, as a plethysmographic chamber and studied the results of chilling the feet and lower legs and of other sensory stimulation, and by Galeotti (1920) who studied the effect upon the temperature of the expired air of cutaneous chilling and warming. Tschalussow found that in a human subject emersion of the feet and lower legs in water at 18° C. caused a marked decrease in the volume of the nasal cavity; electrical stimulation, needle pricks and scratching the skin caused vasoconstriction also, but to a less degree. Galeotti (1914) found that the temperature of the expired air is depressed 1° to 2° C. by cutaneous chilling, raised correspondingly by warming. In explanation of this he advanced the somewhat improbable hypothesis of pulmonary vasoconstriction.

Vasomotor reactions of abnormal tissues.—Reactions in chronic inflammatory throats: One new point of great interest was brought out by the observation experiments in which the pillars and tonsils were seen to blanch with the cutaneous chilling. The pharynx of this same subject was much injected. He gave a history of having noticed a sore throat about a week before, which he thought had cleared up; the night before the experiment he had driven an open machine without an overcoat, and his sore throat had returned. The diagnosis was chronic catarrhal pharyngitis with pustules. This inflamed throat did not pale with the rest of the mucous membrane, but if anything, its injection was intensified by chilling the body surface.

In a thermogalvanometric experiment upon a case of chronic pharyngitis of two years' standing, a similar reaction resulted (Fig. 13).

History.—Began smoking 1915; used about four cigarettes daily. In 1916, began smoking fifteen to twenty cigarettes daily; about this time pharyngitis began. Since has smoked eight to ten cigarettes daily. Treated April, May and June, 1917, Johns Hopkins dispensary, with argyrol, etc. No improvement. Treated twice a week with argyrol and silver nitrate, November and December, 1918, in St. Louis. No improvement. Told smoking was probable cause, and if he would stop smoking throat would get well. Condition unchanged August 2, 1918, when experiment was performed.

EXPERIMENT G.—M.M. thermopile on *inflamed posterior wall of oropharynx.* Skin thermopile on forehead. Respiration, 13 per minute. Nostrils plugged; mouth breathing. Thoracic and abdominal pneumographs. Room temperature, 18.7° to 19.5° C.; time, 10:10 to 11:35 P.M.

The skin curve in Fig. 13 is typical. The mucous membrane temperature shows the fall with deepened respiration which of necessity would follow inhalation into the pharynx through the open mouth of increased volume of cold air. However, with chilling, 0:18.5 to 0:42, the mucous membrane temperature, instead of dropping with the skin temperature, shows a slight transient rise. The shape of the curve, reaching its height in the middle of the period of chilling and then slowly sinking, apparently uninfluenced by the cessation of chilling, suggests that it records some slight changes in local vasomotor tone quite independent of the cutaneous chilling, or alterations in general blood pressure, or some slight accidental change in experimental conditions. At all events there is no questioning the fact that the normal reflex vasoconstriction is absent.

Inhalation of an ampule of amyl nitrite (1:08 to 1:09.5) was followed by characteristic vasodilation. Measurement of the respiration record shows breathing to have been suddenly deepened at 1:09 (Fig. 13, *r*) and to have maintained itself abnormally deep until 1:14.5, when the subject could no longer maintain the slow rhythm, and respiration became quick and shallow. The preliminary fall of mucous membrane temperature from 1:09 to 1:10.5 was

doubtless due, therefore, to deepened respiration and a fall in general blood pressure; the rise from 1:10.5 to 1:13.25 was unquestionably due to local vasodilation and occurred in spite of deepened respiration and lowered blood pressure.

Amyl nitrite causes vasodilation by direct action upon the smooth muscle of the blood vessel walls. This typical dilator response of the inflamed throat to amyl nitrite shows, therefore, that the vessel walls are capable of reacting normally. The typical skin curve would indicate proper functioning on the part of the afferent and association elements of the reflex arc. This failure of normal reaction to cutaneous chilling, in so far as conclusions can be drawn from a single experiment, must then be referred to the motor elements of the reflex arc, and probably has its seat in or near the inflamed mucous membrane.*

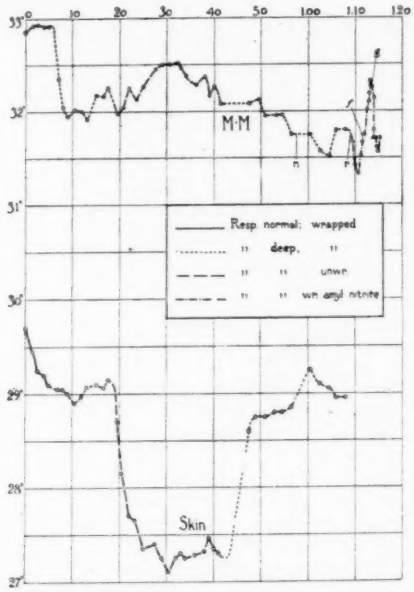


Fig. 13.—Chilling and amyl nitrite effects upon chronically inflamed oropharynx. Experiment G (temperatures of skin and mucous membrane of oropharynx): *n*, plugs pulled from subject's nose; begins nose breathing; *r*, respiration suddenly deepened; *f*, face flushed, *g*, flush passing; respiration still exaggerated.

Reaction to amyl nitrite of an acute inflammatory palate:

EXPERIMENT H.—M.M. thermopile upon the *intensely injected soft palate.* Acute pharyngitis and tonsillitis. Respiration uncontrolled. Mouth breathing (except 0:00 to 0:23).

At 0:10.5 (Fig. 14) subject inhaled by mouth an ampule of amyl nitrite. A steep fall of mucous membrane temperature amounting to 2.69° C. followed. The minimum temperature was reached in 13.5 minutes, and was followed by a rise of 2.56° C., attained in four minutes.

Evidently the vessels of the inflamed membrane were practically maximally dilated. The temperature change observed was therefore the result of the increase in depth of respiration and the depression of general blood pressure.

Reflex reactions of scar tissue to chilling:

*This experiment is included as suggestive merely, not conclusive; for in later work it was found that a normal throat which ordinarily responded to chilling by reflex vasoconstriction would occasionally fail to exhibit that response. The explanation seemed to be usually or always that excessive swallowing and resultant traumatization of the pharynx occurred during the period of chilling, with consequent dilation of the vessels, as shown in Fig. 6. Such may possibly have been the case in this experiment.

EXPERIMENT I.—Keloid removed from chest wall August 24, 1918. Experiment performed September 25, 1918. Scar at that time 2.5 by 1 cm.; red; covered with epithelium. First thermopile on normal skin of chest near scar. Second thermopile on scar. Room temperature, 21.35° to 21.6° C. (See Fig. 15, curves on left.)

The subject sat wrapped from 0:00 to 0:11.5; the sites of application of the thermopiles were of course freely exposed. At 0:11.5, the subject was bared from the waist up, and chilled with ice bags to her back. Skin and scar temperatures both fell sharply. The scar temperature reached a maximum depression of .90° C. in 1.0 minute, the skin of 1.12° C. in 4.5 minutes. At 0:17.5, the subject gasped and heaved her chest. Possibly as a result of mechanical irritation, the scar temperature at once rose, although skin tem-

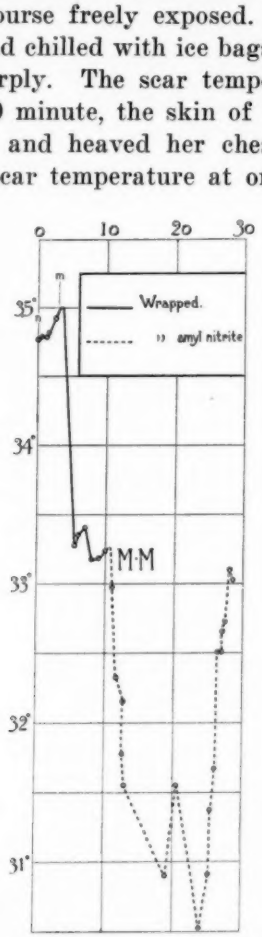


Fig. 14.—Effect of amyl nitrite upon acutely inflamed soft palate. Experiment H (temperatures of mucous membrane of soft palate): *n*, breathing through nose; *m*, changes to mouth breathing.

perature was not affected. At 0:20.75 the subject stood up to wrap herself, and may have disturbed the position of the scar applicator, although no displacement was visible. After wrapping, the skin temperature rose typically, the scar temperature fell.

This experiment on new scar tissue shows, certainly, reflex vasoconstriction in response to chilling. To what degree the later atypical behavior of the scar temperature actually represents its vasomotor reactions and to what the failure of the subject to coöperate, it is difficult to say.

EXPERIMENT J.—Radical operation June 7, 1918, for carcinoma of breast. Experiment performed September 23, 1918. First thermopile on normal skin near scar. Second thermopile breast. Sites of application exposed throughout experiment. Room temperature, 15.65°-16.35° C. (See Fig. 15, middle curves.)

The subject was chilled at 0:07.5 merely by unwrapping in the cold room. Scar temperature dropped 1.80° C. within 5.0 minutes; skin temperature, 2.63° C. within 5.0 minutes. Upon rewrapping at 0:14.5, scar temperature rose .45° C. in 3.0 minutes; skin temperature, 1.54° C. in 4.0 minutes.

EXPERIMENT K.—Admitted to hospital, February 1, 1918, with history of having ulcer on forehead for a year. Diagnosis; syphilitic ulcer. Slow improvement under specific treatment. June 21, 1918, skin graft to ulcer from leg. Favorable. Discharged July 7, 1918. Experiment performed September 12, 1918. Scar about 5 cm. in diameter, red, covered with thin epithelium. First thermopile on normal skin near scar. Second thermopile on scar. Room temperature, 17.8° to 17.95° C. (See Fig. 15, curves on right.)

At 0:11.5 the subject was bared from the waist up, and an electric fan turned on her back. Hairs over her forehead were not disturbed; thus the sites of application were not much cooled by draft. The scar temperature fell

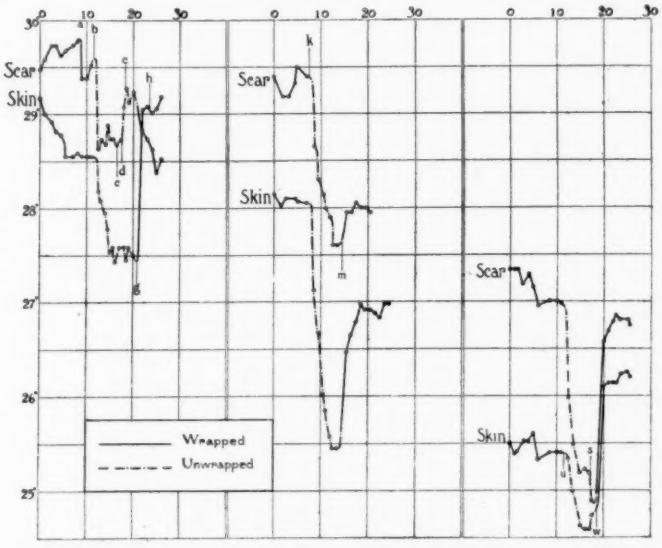


Fig. 15.—Reflex effects of chilling on scar tissue. Experiments I, J, and K (temperatures of scars and contiguous skin): Experiment I: *a*, subject moves, sighs, etc.; *b*, subject bared from waist up; ice bags to back; *c*, gasps; *d*, gasps, heaves chest; *g*, wrapped (subject stood up to wrap self; when she sat down again the skin applicator was found to have tilted; the scar applicator seemed all right, but it also had probably moved somewhat): *h*, heaves sigh. Experiment J: *k*, body bared from waist up; no fan or ice bag used; *m*, wrapped. Experiment *K*: *u*, body bared from waist up; fan on back; hairs on forehead not disturbed at all by draft; *s*, shivers; *w*, fan off; wrapped.

2.09° C. in 6.0 minutes, the skin 0.82° in 4.5 minutes. Upon wrapping at 0:18.5, scar temperature rose 1.96° C. in 4.0 minutes; skin 1.55° C. in 6.5 minutes.

These reactions would seem to explain the cyanosis of fresh scars during cutaneous chilling. The arteriolar constriction doubtless renders the flow of blood through the wide capillary network scant and sluggish, thus allowing unusually complete absorption of oxygen from the oxyhemoglobin.

AFTER EFFECTS OF CHILLING-EXPERIMENTS OF SERIES OF 1918

Subject S. B. G. after being chilled as subject for fourteen experiments over a period of twenty-two days in midsummer, developed severe rhinitis, followed quickly by pharyngitis, tonsillitis, and injection of uvula and soft palate. Sub-

ject retained an abnormal susceptibility to colds throughout fall and early winter.

Subject S. M., after being chilled as subject for fifteen experiments over a period of twenty-four days in midsummer, developed a slight neuralgia in left shoulder. Next day was again subject. The day thereafter pleurisy developed in left chest. Neuralgia yielded to local treatment. Pleuritic symptoms lasted two weeks. Mucous membranes remained normal.

Chilled again in November without ill effect. Chilled December 4; experiment 4:00 to 6:00 P.M. By following morning slight congestion in nasopharynx had developed, which persisted three or four days. Chilled January 2 without after-effect other than nausea, vomiting and headache.

This subject noticed that entering the cold room from the hot outside air of summer was frequently followed by violent intestinal peristalsis and defecation. This subject irrigated his nose and throat after each exposure with a weakly alkaline salt solution, used *hot*, with the idea of both cleansing and of producing hyperemia. Following irrigation a spray of 0.5 per cent phenol in liquid albolene was used after the winter exposures.

Subject A. G., on August 1, chilled. After exposure, low grade pharyngitis developed and persisted some days.

Subject J. D. R., with chronic pharyngitis; chilled twice, subject thought after second exposure was exacerbation of pharyngitis.

Subject G. A., suffered after single exposure no ill effects other than headache.

Subject B. J. F., no symptoms after an observation experiment.

CONCURRENT BACTERIOLOGIC STUDIES—SUMMER OF 1919

Several instances during the experiments of 1918 seemed at least suggestive of experimental excitation of infection. The case of S. M. in particular seemed to point to chilling as the exciting factor. The experiment was a blood temperature control in which he sat from 4 to 6:09 P.M., December 4, 1918, with closed mouth, the bulb of a thermometer beneath his tongue. He forced respiration from 4:12 to 6:09 P.M., and was chilled by a fan from 4:47 to 5:09 P.M. Shivering began at 4:48 P.M., and had become very severe by 4:52 P.M. The subject had not been aware of other recent exposure to cold or infection. By the morning of December 5, nasopharyngeal stuffiness had developed sufficiently to cause the remark by a friend that he had a cold. The symptoms persisted three or four days.*

A study of the flora of the nose and throat during the experiments of the summer of 1919 seemed, then, to be well worth while.

Material and Method.—The medium employed was a 5 per cent rabbit blood meat infusion agar. Baked blood agar was also used in each instance as a special medium for Bacillus influenzæ. Sputum from each subject was injected into a mouse for typing pneumococcus. Cultures were taken from the nose through the anterior nares, from the tonsil, and from the posterior pharyngeal wall by means of separate swabs. Each swab was immersed in

*Similarly we have noted what we believe to be an excitation of infection after, and presumably due to chilling in a number of carefully observed instances in our everyday experience.

sterile broth and then applied both to a red and baked blood agar plate, the remaining area of the plate was inoculated by means of a platinum loop. Films were made directly from the same swab.

The cultures were incubated for 36 hours. The plates were then divided into eight segments, and every colony in two to four segments of the plate was counted and its nature determined. An attempt was made to detect any marked changes in the flora and particularly in the relative proportions of the bacteria present. On account of the difficulty of differentiating pneumococcus from Streptococcus viridans by morphology these two were put in the same group.

Results.—Four different subjects were used in this study. Two developed clinical sore throats; a third had some symptoms of malaise and headache; a fourth was unaffected. The results obtained with the nose cultures were entirely negative. Subjects A. G. and S. M. showed Staphylococcus aureus; subjects W. G. E. and S. B. G., Staphylococcus albus. No attempt was made to sterilize the vestibule of the nose before swabbing. The flora obtained from the nose, as has been found by other investigators (Thomson, 1913), was at all times exceedingly sparse, but the above organisms were always found to be present. They showed no changes with exposure. The results obtained from pharynx and tonsil in each instance are given in Tables I to III.

From the pharynx and tonsil of Subject S. M. before the experiment there were cultured Streptococcus viridans, Pneumococcus Type II atypical, and Bacillus influnzæ. He was then subject of Experiment 7. Within 24 hours he had a clinical sore throat; coincident with this, there was a sudden appearance of Streptococcus hemolyticus in the cultures from both tonsil and pharynx. During the days following, with the disappearance of the sore throat, the number of colonies of Streptococcus hemolyticus fell off rapidly. The remaining bacteria showed no evident change.

EXPERIMENT 7.—Subject S. M., June 7, 1919, 3:45 to 6 P.M. Mucous membrane thermopile on left tonsil. Respirations 14 per minute. Mouth open; nostrils plugged. Room temperature 18.10-19.60° C. 0:00 to 0:31.5. Wrapped; normal breathing; swallowed many times. 0:31.5 to 0:44. Wrapped; deep breathing. 0:44 to 0:55.5. Unwrapped; fan on back; deep breathing. 0:52. Shivers. 0:55.5. Coughs, chokes, applicator removed; blood flecks seen about terminals. 4:57 P.M., experiment started again. 0:00 to 0:06. Wrapped; deep breathing. 0:06 to 0:13.5. Unwrapped; fan on; deep breathing; shivering; swallows many times. 0:13.5 to 0:33.5. Wrapped; deep breathing. 0:33.5. Conditions the same; amyl nitrite inhaled. 0:35 to 0:44. Wrapped; deep breathing, some coughing and swallowing. 0:44 to 0:53. Hot water bag around subject; wrapped; deep breathing.

On the following morning the feeling of soreness had practically left the traumatized tonsil, but the posterior wall of the oropharynx felt sore. On inspection a localized area of injection bearing a whitish exudate was seen on the posterior wall of the oropharynx. In culturing, the contact of the swab on this area was a little painful, and the culture yielded streptococci, as explained above. The tonsillar ring was injected, but the feeling of soreness on the tonsil entirely passed off during the day and contact of the swab in culturing was hardly felt. Thus the traumatized tonsil showed less evidence of being the site of an active infection than the posterior pharyngeal wall, which was thought not to have been directly traumatized, but of course we cannot ex-

TABLE I

SUBJECT S.M. BACTERIOLOGY OF TONSIL AND PHARYNX

DATE	PLACE CULTURED	*S. viridans and Pn. II*		*S. hæmolyticus.*		*B. influenzæ*		UNDETERMINED		REMARKS
		NO. OF COLONIES COUNTED	PER CENT OF ALL COLONIES	NO. OF COLONIES COUNTED	PER CENT OF ALL COLONIES	NO. OF COLONIES COUNTED	PER CENT OF ALL COLONIES	NO. OF COLONIES COUNTED	PER CENT OF ALL COLONIES	
1919										
June 4	Left tonsil	28	93	0	0	2	7	0	0	
	Pharynx	36	100	0	0	0	0	0	0	
						Present in baked blood				
" 5	Left tonsil	43	100	0	0	" "	" "	0	0	
	Pharynx	31	94	0	0	" "	" "	4	6	
" 6	Left tonsil	35	100	0	0	" "	" "	0	0	
	Pharynx	24	86	0	0	" "	" "	4	14	
" 7*	Left tonsil	52	94	0	0	3	6	0	0	Subject of experiment. Application on left tonsil. Cultures after experiment.
	Pharynx	40	87	0	0	4	13	0	0	
" 8	Left tonsil	57	65	13	15	7	8	11	12	Subjective sore throat. Pharynx shows injection with white exudate; tonsillar ring injected.
	Pharynx	26	56	14	31	6	13	0	0	
" 9	Left tonsil	35	76	6	13	1	2	4	8	Very slight soreness. Throat no longer injected.
	Pharynx	34	79	9	21	Present in baked blood		0	0	
" 11	Left tonsil	34	94	1	3	1	3	0	0	
	Pharynx	30	91	3	9	Present in baked blood		0	0	Throat normal.

*Experiment 7.

TABLE II

SUBJECT W. G. E. BACTERIOLOGY OF TONSIL AND PHARYNX

DATE	PLACE CULTURED	*S. viridans*		*S. hæmolyticus*		*B. influenzæ*		*S. albus*		REMARKS
		NO. OF COLONIES COUNTED	PER CENT OF ALL COLONIES	NO. OF COLONIES COUNTED	PER CENT OF ALL COLONIES	NO. OF COLONIES COUNTED	PER CENT OF ALL COLONIES	NO. OF COLONIES COUNTED	PER CENT OF ALL COLONIES	
1919										
June 10	Left tonsil	18	42	4	9	9	21	12	28	
	Pharynx	13	52	0	0	12	48	0	0	
" 11*	Left tonsil	28	68	3	8	8	20	2	4	Subject of experiment. Application on left tonsil. Cultures after experiment.
	Pharynx	24	72	0	0	6	18	3	9	
" 12	Left tonsil	31	55	3	5	5	9	17	31	No after affects.
	Pharynx	18	54	0	0	12	36	4	9	
" 16†	Left tonsil	26	53	0	0	6	12	18	35	Subject of experiment. Application on soft palate. Cultures taken before experiment.
	Pharynx	32	60	0	0	9	17	12	23	
" 17	Left tonsil	14	45	2	7	8	26	7	22	Subject complains of general malaise; slight headache; no sore throat. Film from swab shows mostly Gram-negative bacilli with a few cocci.
	Pharynx									
	Red blood	0	0	0	0		100	0	0	
	Baked blood	0	0	0	0		100	0	0	
" 19	Left tonsil	19	56	2	6	5	15	8	24	
	Pharynx	21	60	0	0	14	40	0	0	
" 20	Left tonsil	21	50	3	7	7	17	11	26	
	Pharynx	19	58	0	0	11	33	3	9	

*Experiment D.
†Experiment 8.

TABLE III

SUBJECT A.G. BACTERIOLOGY OF TONSIL AND PHARYNX

DATE	PLACE CULTURED	*S. viridans*		*M. catarrhalis*		*S. aureus*		*S. albus*		REMARKS
		NO. OF COLONIES COUNTED	PER CENT OF ALL COLONIES	NO. OF COLONIES COUNTED	PER CENT OF ALL COLONIES	NO. OF COLONIES COUNTED	PER CENT OF ALL COLONIES	NO. OF COLONIES COUNTED	PER CENT OF ALL COLONIES	
1919										
June 7	Left tonsil	29	85	0	0	0	0	5	15	
	Pharynx	21	91	0	0	0	0	2	9	
" 8*	Left tonsil	49	90	0	0	0	0	5	10	Subject of experiment. Application on left tonsil. Cultures after experiment
	Pharynx	27	75	0	0	2	6	7	19	
" 9	Left tonsil	21	60	3	9	0	0	11	31	No symptoms
	Pharynx	31	57	15	27	1	2	7	14	
" 10†	Left tonsil	27	52	4	8	2	4	19	36	Subject of experiment. Application on right tonsil. Cultures after experiment
	Pharynx	34	81	0	0	3	7	5	12	
" 11	Left tonsil	34	85	1	2	0	0	5	13	
	Pharynx	39	93	0	0	0	0	3	7	
" 12	Right tonsil	30	73	0	0	4	10	7	17	
	Pharynx	34	87	2	5	1	3	2	5	
" 13‡	Right tonsil	40	88	2	5	0	0	3	7	Subject of experiment. Application on pharynx. Cultures after experiment
	Pharynx	27	70	7	19	0	0	4	11	
" 14	Right tonsil	21	58	12	34	0	0	3	8	
	Pharynx	15	45	4	11	11	39	2	5	
" 15										No cultures. Subject has soreness on swallowing.
" 16§	Right tonsil	21	52	11	27	0	0	8	20	Subject of experiment. Application on
	Pharynx	29	60	15	31	0	0	4	9	pharynx. Marked congestion of tonsillar ring and pharynx
" 17	Right tonsil	34	72	3	7	8	17	2	4	Throat injection milder. No soreness on
	Pharynx	27	75	6	16	3	8	0	0	swallowing
" 18‖	Right tonsil	25	59	2	5	15	36	0	0	Subject of experiment. Application on
	Pharynx	31	77	4	10	5	13	0	0	soft palate. Cultures after experiment
" 19	Right tonsil	28	54	15	28	9	18	0	0	Soreness on swallowing. Injection of ton-
	Pharynx	32	64	11	22	7	14	0	0	sillar ring
" 20										No cultures. Sore throat disappeared
" 21	Right tonsil	35	83	0	0	4	10	3	7	No soreness. No injection
	Pharynx	31	93	1	3	0	0	1	3	
" 23	Right tonsil	31	81	2	5	1	2	4	11	No soreness. No injection
	Pharynx	27	81	1	3	2	6	3	9	

*Experiment 9. †Experiment 6. ‡Experiment 10. §Experiment 11. ‖Experiment 12.

clude the possibility that the oropharynx was infected by hemolytic streptococci from the tonsillar crypts—so commonly a habitat for them—which were missed in the earlier cultures and were disseminated by the experimental trauma and swallowing.

In subject W. G. E. there were present Streptococcus viridans, Bacillus influenzæ, Streptococcus hemolyticus, and Staphylococcus albus. Following Experiment D there were no noteworthy changes. The plate inoculated from the pharynx about 26 hours after Experiment 8, however, showed a pure culture of Bacillus influenzæ; the tonsillar plate showed also a slight relative increase. The film from the pharynx showed practically all Gram-negative bacilli with an occasional coccus. The subject had no sore throat, but complained of general malaise, slight headache, and some chilly sensations.* The pharynx culture made 96 hours after Experiment 8 was practically the same as before the experiment, Streptococcus viridans and Staphylococcus albus appearing as before.

EXPERIMENT 8.†—Subject W. G. E., June 16, 1919, 3:20 to 4:07 P.M. Mucous membrane thermopile on anterior half of soft palate, left side. Respirations 16 per minute. Mouth open; nostrils plugged. Room temperature 20.22-20.90° C. 0:00 to 0:05.5. Wrapped; normal breathing. 0:05.5 to 0:11. Wrapped; deep breathing. 0:11 to 0:32. Unwrapped; fan on back; deep breathing. 0:32 to 0:47. Wrapped; deep breathing.

With the applicator resting against the anterior soft palate there is no reason for supposing trauma to the tonsils and pharynx. Organisms could hardly have been introduced from outside by the applicator, for this, with the thermopile terminals attached, was freshly coated with shellac in alcoholic solution before being adjusted for an experiment.

Subject A. G. was the subject of five experiments, June 8, 10, 13, 16, and 18. On June 15 he noted a soreness on swallowing, and on June 16 the entire posterior pharynx and tonsillar ring were distinctly injected. The experiment of June 16 produced no sudden increase in symptoms; on June 17 the sore throat had practically disappeared. On June 19, 36 hours following an experiment, the subject again developed a soreness on swallowing, with congestion of the posterior pharynx and tonsillar ring. On June 20 the symptoms had subsided. On June 21 there was no longer any injection or soreness.

This subject had present in his throat Streptococcus viridans, Staphylococcus aureus, and Staphylococcus albus. Twenty-four hours following the first experiment there was noted for the first time the appearance of Micrococcus catarrhalis. Subsequently there appeared to be a certain degree of correlation between the appearances of sore throat and the rises in relative numbers of Micrococcus catarrhalis colonies. No other change in the bacterial flora was apparent.

EXPERIMENT 9.—Subject A. G., June 8, 1919, 3:38 to 5:06 P.M. Mucous membrane applicator on left tonsil. Respirations 10 per minute. Mouth open; nostrils plugged. Room temperature 18.1-19.45° C. 0:00 to 0:06. Wrapped; normal breathing. 0:06 to 0:15. Wrapped; deep breathing. 0:15 to 0:43. Unwrapped; fan on back; deep breathing; some swallowing, coughing, and clearing of throat; after 0:17, shivering, 0:43 to 1:20. Wrapped; deep respiration. 1:07. Inhales amyl nitrite. 1:20 to 1:28. Hot water pad to back; deep respiration.

The possibility that trauma to the tonsil was responsible for the appearance of Micrococcus catarrhalis after this experiment cannot be excluded.

*Compare the effect of infecting monkeys with B. influenzæ (Blake and Cecil, 1920).

†For Experiment D, see p. 259.

EXPERIMENT 6.—Subject A. G., June 10, 1919, 11:30 A.M. to 12:17 P.M. Mucous membrane thermopile on right tonsil. Skin thermopile on forehead. Respirations 16 per minute. Mouth open; nostrils plugged. Room temperature 16.9°-17.8° C. 0:00 to 0:06.5. Wrapped; normal breathing. 0:06.5 to 0:18.5. Wrapped; deep breathing. 0:18.5 to 0:32. Unwrapped; fan on back; deep breathing; after 0:23.5. Shivering. 0:32 to 0:47. Wrapped; deep breathing; contraction of pharyngeal muscles.

EXPERIMENT 10.—Subject A. G., June 13, 1919, 3 to 3:46 P.M. Mucous membrane applicator on posterior wall of oropharyux. Respirations 16 per minute. Mouth open; nostrils plugged. Room temperature 18.45-19.05° C. 0:00 to 0:04.5. Wrapped; normal breathing. 0:04.5 to 0:15. Wrapped; deep breathing. 0:15 to 0:26. Unwrapped; fan on back; deep breathing; coughed; cleared throat; contraction of pharyngeal muscles; after 0:22, shivers. 0:26 to 0:46. Wrapped; deep breathing; coughed and cleared throat several times; pharynx appeared normal.

EXPERIMENT 11.—Subject A. G., June 16, 1919, 10:30 to 11:30 A.M. Mucous membrane applicator on posterior pharyngeal wall. Respirations 14 per minute. Mouth open; nostrils plugged. Room temperature 18.90°-19.80° C. 0:00 to 0:04.5. Wrapped; normal breathing; contractions of pharyngeal muscles. 0:04.5 to 0:23.5. Wrapped; deep breathing. 0:23.5 to 0:32. Unwrapped; fan on back; deep breathing. 0:32 to 1:00. Wrapped; deep breathing; contractions of pharyngeal muscles.

EXPERIMENT 12.—Subject A.G., June 18, 1919, 10:20 to 11:12 A.M. Mucous membrane applicator on soft palate, middle part. Respirations 14 per minute. Mouth open; nostrils plugged. Room temperature, 18.75°-19.55° C. 0:00 to 0:05.5. Wrapped; normal breathing. 0:05.5 to 0:15.5. Wrapped; deep breathing. 0:15.5 to 0:27. Unwrapped; fan on back; deep breathing. 0:27 to 0:52. Wrapped; deep breathing.

In subject S. B. G. there were present in the throat Streptococcus viridans, Pneumococcus Type IV, and Staphylococcus albus. Cultures were made daily from June 4 to 19; he was the subject of an experiment on June 6, 9, 14, and 17. There was practically no change in the bacterial flora of this subject throughout the entire period studied. Neither did subjective or objective signs of sore throat or cold develop. Frequently the cultures from the pharynx were almost sterile, there being from three to ten colonies over the entire plate.

DISCUSSION OF BACTERIOLOGIC RESULTS

Streptococcus viridans was found in all four of the individuals studied, Bacillus influenzæ in two, pneumococcus in two, and Micrococcus catarrhalis in one subject.

In subject S. M. the increased number of Streptococcus hemolyticus was definitely synchronous with the presence of a sore throat. There appears to have been a correlation between the high Micrococcus catarrhalis counts and the presence of sore throats in Subject A. G. The pure culture of Bacillus influenzæ in the pharynx of Subject W. G. E. was not coincident with sore throat, but with malaise, slight chilliness, and headache.

These results in no sense prove, however, that the sore throats were caused by the increased number of bacteria cultured from the mucous membranes, or that the apparent increase of microorganisms was caused by the ischemia of the mucous membranes incident upon chilling of the body surface. The method is subject to so many sources of error, and the amount of data thus far obtained is so small, that we do not feel justified in drawing any conclusions. To attribute the apparent proliferation of pathogenic microorganisms to the effect of chilling would seem to be in harmony with the great wealth of clinical and

common observation which points to excessive chilling, under proper circumstances, as an efficient excitant of infection of the mucous membranes by their indigenous pathogenic bacteria. Although it is possible that the apparent proliferation was due to the local ischemia incident upon chilling, the inaccuracy of the bacteriologic method and the insufficient data make it impossible to assume that this is so. The effect of trauma by the thermopiles, the possibility of transient changes in the flora of the mucous membranes caused by swallowing, gagging, or other muscular activity in the pharynx pressing a plug of bacteria from the tonsillar crypts, the fact that the subject's mouth was held open throughout the experiments, with the accompanying accumulation of mucus on the membranes, the errors necessarily introduced in each stage of making the cultures, and the inaccuracy of any method depending upon swab cultures, all tend to confuse the results. We present the data given above as a contribution to the etiology of upper respiratory infections, and not with the idea that the study is in any sense complete in itself.

LABORATORY METHODS

REPORT ON FIVE THOUSAND BLOODS TYPED USING MOSS'S GROUPING*

By William L. Culpepper, B.Sc., Dr.P.H., M.D., and Marjorie Ableson, Detroit, Mich.

THERE is at present one of two methods usually employed in typing blood, namely, that of testing the recipient's blood against the donor's *directly* and the *grouping* method. The second method mentioned was the one used in this series of five thousand tests. Until 1901 when a blood transfusion was carried out there were no blood tests made of donor or recipient for hemolysis or agglutination. A number of cases of transfusion with disastrous reactions were reported. This was responsible for so few transfusions prior to that date.

Landsteiner (Ueber Agglutinationsercheinungen Normalen Menschlichen Blutes, in *Wiener Klinische Wochenschrift,* 1910, xiv, 1132) classified all human sera into three groups claiming that all human serum when typed would fall into one of these groups. He designated his groups as follows: Groups A, B, and C.

Group A

Group A Serum agglutinates Corpuscles of Group B.
Group A Corpuscles agglutinated by Serum of Groups B and C.

Group B

Group B Serum agglutinates Corpuscles of Group A.
Group B Corpuscles agglutinated by Serum of Groups A and C.

Group C

Group C Serum agglutinates Corpuscles of Groups A and B.
Group C Corpuscles not agglutinated by Serum of Groups A and B.

This grouping was accepted by the profession until 1910 when Moss began his investigation. In 1907, Jan Jansky (in the review of a paper entitled "Haematologische Studien bei psychotikem," Klincky Sbornik. No. 2, 1907, in *Jahresbericht für Neurologie and Psychiatric*) classified human sera into four groups. In 1910, W. L. Moss (Johns Hopkins Hospital Bulletin, 1910, xxi, 63) pointed out the fallacy in Landsteiner's grouping, which can be seen if compared with Jansky's or Moss's and he (Moss) reclassified them making four groups. Moss's grouping is as follows:

Group I

Group I Sera agglutinate no corpuscles.
Group I Corpuscles agglutinated by sera of Groups II, III and IV.

*From the Research Laboratory of Parke, Davis & Co., Detroit, Mich.

GROUP II

Group 11 Sera agglutinate corpuscles of Groups I and III.
Group II Corpuscles agglutinated by Sera of Groups III and IV.

GROUP III

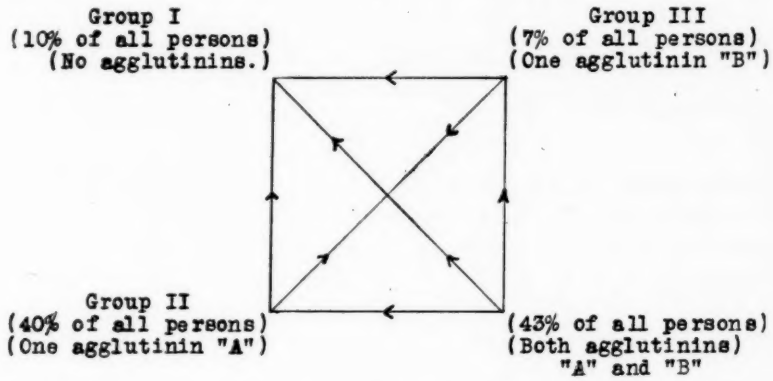
Group III Sera agglutinate corpuscles of Groups I and II.
Group III Corpuscles agglutinated by Sera of Groups II and IV.

GROUP IV

Group IV Sera agglutinate corpuscles of Groups I, II, and III.
Group IV Corpuscles agglutinated by no serum.

The only difference between Jansky's and Moss's grouping, is Jansky's Group I corresponds to Moss's Group IV and vice versa. This grouping is being followed at the present time and forms the basis of this report

As will be noted, Group I contains no isoagglutinins or isohemolysins. Group II contains one agglutinin designated "A." Group III contains one agglutinin designated "B." Group IV contains two agglutinins "A" and "B."



Moss's investigations based on 1600 tests showed that 10 per cent of all persons belong to Group I, 40 per cent to Group II, 7 per cent to Group III and 43 per cent to Group IV.

Our investigations, based on 5,000 tests are recorded in this preliminary report. The ultimate plan being to type 10,000 or more sera. We obtained somewhat different results when compared to Moss's investigations. The sera used were from adults of all ages, from all classes of society, white and negro races. Of the 5,000 examined, 256, or 5.18 per cent were found to belong to Type I; 1805, or 35.06 per cent, to Type II, 714, or 14.28 per cent, to Type III; and 2,225, or 44.48 per cent, to type IV.

The technic of the test is as follows: Two drops of blood containing known Type II corpuscles were suspended in 1 c.c. of a 1.5 per cent solution sodium citrate in 0.9 per cent sodium chloride. A like suspension of Type III corpuscles was also made. A clean sterile glass slide was marked with the name or number of the serum to be typed. With a small sterile pipette two drops of unknown serum to be tested were placed on the slide, one on the left and one on the right. With another sterile pipette, one drop of the suspension of Group II corpuscles was mixed with the serum on the left. With another sterile pipette one drop of

MOSS'S TABLE

First 1,600 tested = 10% Group I, 40% Group II, 7% Group III, 43% Group IV

OUR TABLE

First	1000 tested =	5.2% Group I,	36.0% Group II,	11.7% Group III,	47.1% Group IV
Second	1000 tested =	5.0% Group I,	36.3% Group II,	13.5% Group III,	45.2% Group IV
Third	1000 tested =	4.7% Group I,	35.2% Group II,	15.6% Group III,	44.5% Group IV
Fourth	1000 tested =	5.5% Group I.	36.5% Group II,	15.0% Group III,	43.0% Group IV
Fifth	1000 tested =	5.5% Group I,	36.3% Group II,	15.6% Group III,	42.6% Group IV
Total	5000	25.9%	180.3%	71.4%	222.4%
	5 /	25.9% Group I,	180.3% Group II,	71.4% Group III,	222.4% Group IV
Average per	1000	5.18%	36.06%	14.28%	44.48%

the Group III suspension was mixed with the serum on the right, every precaution being taken to avoid mixing corpuscles of Group II with Group III. Observations were taken every 3 minutes for one-half hour until reaction was complete. Reactions were interpreted as follows:

1. If no agglutination takes place within 30 minutes the serum belongs to Group I, since Group I serum agglutinates the corpuscles of no other group. Members of this group are called "universal recipients," as they may receive blood transfusions from their own group or any other group. They may be donors for members of their own group only.

2. If the corpuscles of Group III are agglutinated and the corpuscles of Group II remain unchanged, the serum belongs to Group II because Group II serum agglutinates the corpuscles of Groups I and III but not the corpuscles of its own group or those of Group IV. Members of this group may receive blood transfusions from their own group or from Group IV. They may be donors for their own group or Group I.

3. If the corpuscles of Group II are agglutinated and the corpuscles of Group III remain unchanged the serum belongs to Group III because Group III serum agglutinates the corpuscles of Groups I and II but not those of its own group or of Group IV. Members of this group may receive blood transfusions from their own group or from Group IV. They may be used as donors for their own group or to Group I.

4. If the corpuscles of Group II and Group III both show agglutination, the serum belongs to Group IV because Group IV serum agglutinates the corpuscles of all other groups. Members of this group may receive blood transfusions only from members of their own groups. They may be used as donors for Groups I, II, III and IV and are termed "universal donors."

Agglutination and hemolysis may be observed at any time from 60 seconds up to 30 minutes or until the serum evaporates. Hemolysis does not occur without agglutination, but agglutination may occur without hemolysis. Where agglutination only takes place, the corpuscles impart a finely granular appearance to the field. This may usually be seen macroscopically, but can be more readily determined in doubtful cases with the aid of a microscope. Where hemolysis and agglutination both occur the cells are gathered into red clumps or ropes clearly discerned macroscopically. It is possible to determine the group to which an

individual belongs after the slide has dried. Therefore, the slide may be filed away as a permanent record if so desired.

The possibilities that all groups may contain both agglutinins A and B, but in varying amounts or strengths, was suggested by the following observation: A serum giving a marked reaction for a specific group may show a faint inclination to react to the corpuscles of another group, varying from a barely perceptible to a marked reaction. Example—a known suspension of cells of a Group II plus the unknown serum gives a marked agglutination. A known suspension of cells of a Group III plus unknown serum gives a faintly perceptible agglutination. A known suspension of cells of a Group II plus the second unknown serum gives a marked agglutination. A known suspension of cells of a Group III plus the second unknown serum give a moderate agglutination. A known suspension of cells of a Group II plus the third unknown serum gives a marked reaction. A known suspension of cells of Group III plus the third unknown serum gives a strong agglutination. The same suspension of corpuscles were used in this specific observation throughout, it being considered the constant factor.

Second phase of this observation: fresh sera failing to give a positive agglutination for a specific group of corpuscles, when crossed (diluted) with an equal volume of another unknown serum (so far as we were able to ascertain, of the same group by previous typing) and allowed to evaporate at 35° F., approximately 1/10 its original volume, gave a positive reaction. Example—two sera, each showing a positive agglutination for Group III corpuscles and no reaction with Group II corpuscles, macroscopically, were combined in equal volumes and placed in the ice box at a constant temperature of 35° F. for two days, evaporation being encouraged by removal of the stopper from the container. The combined sera were then tested at the expiration of 48 hours with corpuscles (obtained from the same individuals which were used in the first test) of Groups II and III. Both groups gave a positive agglutination, that of Group III being very decided, and that of Group II not so strongly marked but quite unmistakable. This phase would introduce so many possibilities, namely, biochemical changes of the sera or cells, or both, used, that it may be valueless, but the writer thought it well to mention it. On the other hand, it may explain why there is occasionally a reaction, moderate or severe, when transfusions are carried out, by the sodium citrate method, although the donor and recipient belong to the same group.

Third phase of this specific observation: A Group IV serum may contain more isoagglutinins A than B or vice versa. Example—a Group IV serum was diluted with 1.5 per cent sodium citrate in 0.9 per cent sodium chloride. Tests were made with the following dilutions:

One part serum to one part of the diluent.
One part serum to three parts of the diluent.
One part serum to seven parts of the diluent.
One part serum to fifteen parts of the diluent.
One part serum to thirty-one parts of the diluent.
One part serum to sixty-three parts of the diluent.

The result was:

In 1:1 dilution reaction with Group II corpuscles ++++; with Group III corpuscles ++.

In 1:7 dilution reaction with Group II corpuscles +++; with Group III corpuscles +.

In 1:15 dilution reaction with Group II corpuscles ++; with Group III corpuscles –.

In 1:31 dilution reaction with Group II corpuscles + (with microscope) with Group III corpuscles –.

In 1:63 dilution reaction with Group II corpuscles –+ (partial with microscope) with Group III corpuscles –.

This phase of the investigation was repeated several other times with other sera of Group IV and each time the results checked, that is, the reaction never disappeared from both when using the same dilution.

Fourth phase: The possibilities are that Group I may contain isoagglutinins A, B or both, but in such minute amounts that the reaction, if there is one it is not appreciable.

GROUP IV SERUM

SERUM USED	DILUENT	PROPORTION	USED CORPUSCLES OF GROUP	REACTION	USED CORP. OF GROUP	REACTION
1 part	1 part	1:1	II	Strong	III	Strong
1 "	3 parts	1:3	II	Complete	III	Complete
1 "	7 "	1:7	II	Weak	III	Partial
1 "	15 "	1:15	II	Negative	III	Weak
1 "	31 "	1:31	II	Negative	III	Very faint
1 "	64 "	1:64	II	Negative	III	Negative
1 "	1 part	1:1	II	Strong	III	Strong
1 "	3 parts	1:3	II	Strong	III	Strong
1 "	7 "	1:7	II	Weak	III	Weak
1 "	15 "	1:15	II	Negative	III	Partial
1 "	31 "	1:31	II	Negative	III	Negative

SERUM IV USED IN FIRST AND SECOND GROUP OF TESTS

SERUM USED	DILUENT	PROPORTION	USED CORPUSCLES OF GROUP	REACTION	USED CORP. OF GROUP	REACTION
1 part	1 part	1:1	II	++++++	III	++++++
1 "	3 parts	1:3	II	+++++	III	+++++
1 "	7 "	1:7	II	++++	III	+
1 "	15 "	1:15	II	+++	III	–
1 "	31 "	1:31	II	++	III	–
1 "	63 "	1:63	II	+	III	–
1 "	127 "	1:127	II	–	III	–

SERUM III USED IN SECOND AND THIRD GROUP OF TESTS

SERUM USED	DILUENT	PROPORTION	USED CORPUSCLES OF GROUP	REACTION	USED CORP. OF GROUP	REACTION
1 part	2 parts	1:2	II	++++++	–	–
1 "	5 "	1:5	II	++++++	–	–
1 "	11 "	1:11	II	+++++	–	–
1 "	23 "	1:23	II	++++	–	–
1 "	47 "	1:47	II	+++	–	–
1 "	95 "	1:95	II	++	–	–
1 "	143 "	1:143	II	+	–	–
1 "	179 "	1:179	II	–	–	–

The limitations of dilution of the five thousand sera was fairly constant at a 1:150. Above this dilution we failed to get a reaction. The series were not diluted higher than a 1:9 for grouping the unknown.

Thermal reactions:

First Group of tests.

Group IV serum (undiluted and containing no preservative) tested with corpuscles of Groups II and III, respectively.

At 23° C., reaction strong in both Groups II and III corpuscles.
At 60° C., reaction strong in both Groups II and III corpuscles and much more rapid.
At 65° C., reaction strong in both Groups II and III corpuscles.
At 71° C., serum coagulated making test impossible.

Second Group tests; serum undiluted and containing no preservative.

At 22° C., reaction strong in both Groups II and III corpuscles.
At 60° C., reaction strong in both Groups II and III corpuscles.
At 65° C., reaction strong in both Groups II and III corpuscles.
At 67° C., reaction weak and less rapid.
At 70° C., reaction weak in both Groups II and III corpuscles.
At 72° C., Serum coagulated.

Third group of tests, Type II serum diluted 1:1 and containing 1:250 purified cresols.

At 17° C., strong reaction in Group III corpuscles.
At 60° C., strong reaction in Group III corpuscles and rapid.
At 70° C., strong reaction in Group III.
At 75° C., coagulates.

Fourth group of tests; Type II serum undiluted and containing no preservative.

At 22° C., strong reaction in Group III corpuscles.
At 60° C., strong reaction in Group III corpuscles.
At 71° C., coagulation beginning.
At 72° C., coagulation complete.

Fifth group of tests; Group IV serum undiluted and not preserved.

At 2° C., reaction strong in both Groups II and III but not so rapid as at room temperature.

At 0° C., reaction strong in both Groups II and III but not so rapid as at room temperature.

The serum was then frozen hard, thawed and tested. Reaction was strong and not appreciably retarded.

Influence of preservatives:

A 1:250 purified cresol was used on some Type II and III serum early in March, 1920, and these sera are active on this date. They were kept at ordinary ice box temperature during this time, however. We are now conducting some investigations along this line as to other preservatives.

Influence of syphilis on the reaction:

The table herewith attached represents only sixty-six cases giving a positive Wassermann reaction.

Apparently blood serum that exhibits a positive Wassermann reaction does not change the typing reaction. Moss found this to be the case in 1907.

In typing the number of cases reported here, we selected one thousand sera which had been typed and recorded, then we allowed them to be exposed to the air, which resulted in bacterial contamination. They were then typed again at

REACTIONS WITH POSITIVE WASSERMANN SERA

DATE	SERUM REACTION				GROUP	DEGREE OF REACTION
7/29	+III	corpuscles	and	−II C.	II	Strong
"	+III	"	"	−II C.	II	"
8/4	+III	"	"	+II C.	IV	"
"	+III	"	"	+II C.	IV	"
"	−III	"	"	−II C.	I	"
"	+III	"	"	+II C.	IV	"
"	+III	"	"	+II C.	IV	"
"	+III	"	"	−II C.	II	"
8/5	+III	"	"	−II C.	II	"
"	−III	"	"	+II C.	III	"
"	+III	"	"	−II C.	II	"
"	+III	"	"	+II C.	IV	"
"	+III	"	"	−II C.	II	"
"	+III	"	"	−II C.	II	"
8/7	+III	"	"	+II C.	II	"
"	+III	"	"	−II C.	IV	"
8/10	+III	"	"	+II C.	IV	"
"	−III	"	"	+II C.	III	"
"	+III	"	"	+II C.	IV	"
"	−III	"	"	−II C.	I	"
"	+III	"	"	−II C.	II	"
"	+III	"	"	+II C.	IV	"
8/11	−III	"	"	+II C.	III	"
"	+III	"	"	+II C.	IV	"
"	+III	"	"	−II C.	II	"
"	−III	"	"	+II C.	III	"
"	+III	"	"	+II C.	IV	"
8/13	−III	"	"	+II C.	III	"
"	−III	"	"	+II C.	III	"
"	+III	"	"	−II C.	II	"
"	+III	"	"	+II C.	IV	"
"	−III	"	"	+II C.	III	"
8/14	+III	"	"	−II C.	II	"
"	+III	"	"	−II C.	II	"
"	+III	"	"	+II C.	IV	"
"	+III	"	"	+II C.	IV	"
"	+III	"	"	+II C.	IV	"
"	+III	"	"	−II C.	II	"
"	+III	"	"	+II C.	IV	"
9/22	+III	"	"	+II C.	IV	"
"	+III	"	"	−II C.	II	"
"	+III	"	"	−II C.	II	"
"	+III	"	"	−II C.	II	"
"	+III	"	"	+II C.	IV	"
"	+III	"	"	−II C.	II	"
"	+III	"	"	−II C.	II	"
"	+III	"	"	+II C.	IV	"
"	+III	"	"	+II C.	IV	"
"	+III	"	"	−II C.	II	"
"	+III	"	"	−II C.	II	"
"	+III	"	"	−II C.	II	"
"	+III	"	"	+II C.	IV	"
"	+III	"	"	+II C.	IV	"
"	−III	"	"	+II C.	III	"
"	−III	"	"	+II C.	II	"
"	+III	"	"	+II C.	IV	"
"	−III	"	"	+II C.	III	"
"	+III	"	"	+II C.	IV	"
"	+III	"	"	−II C.	II	"
"	−III	"	"	+II C.	III	"
"	−III	"	"	+II C.	III	"
"	+III	"	"	−II C.	II	"
"	+III	"	"	+II C.	IV	"
"	−III	"	"	+II C.	III	"
"	+III	"	"	+II C.	IV	"
"	+III	"	"	+II C.	IV	"

Total 66

Total	Group	I = 2	or	3.03%
"	"	II = 25	"	37.87%
"	"	III = 12	"	18.18%
"	"	IV = 27	"	40.90%
Total	Groups	= 66		99.98%

the end of six days and we found that they gave the same reaction that they exhibited when first typed. Neither was the time for the reaction to take place altered.

CONCLUSIONS

1. Without exception the entire 5000 sera could be grouped and classified according to Moss.

2. Our group percentage in Group I was 4.9 per cent lower than Moss's; in Group II it was 4.1 per cent lower; in Group III it was 7.2 per cent higher; and in Group IV it was 1.5 per cent higher.

3. A permanent slide record may be kept of a reaction if desired.

4. There is a probability that types may overlap. A reaction in the recipient of blood from a donor of the same type may be explained by this probably overlapping.

5. Hemolysis is not specific for any one group.

6. A 1:10 dilution of a serum is very convenient to work with. The maximum dilution we found to be 1:150. The suspension of 1 drop of blood to 1 c.c. of the citrate salt solution is most convenient to work with.

7. The reaction works best at about 40° C. Heat does not impair the reaction until coagulation takes place (at about 71°C). Cold does not interfere with the reaction, although the serum may have been frozen. Bacterial contamination or purified cresol in a dilution of 1:250 does not vary the reaction. Positive Wassermann sera do not influence the typing reaction.

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EDITORIALS

Roentgen Ray Therapy in Hyperthyroidism

NUMEROUS recent reports of beneficial effects of the roentgen ray and radium in the treatment of hyperthyroidism give evidence of the apparently good results accomplished by the method. While the etiology of the disease remains uncertain, all treatment must perforce be empiric. The malady has been variously ascribed to, (a) psychic influences, such as fear, shock, or other violent emotion; (b) foci of infection, such as tonsils, adenoids, etc.; (c) hyperactivity of other endocrine glands. Possibly the exciting cause differs in different instances, but the predisposing cause is presumably very similar in all cases.

Under medical treatment the symptoms of hyperthyroidism may be relieved, but seldom does the disease entirely disappear within the period of observation. Surgical operation, preceded and followed by prolonged medical care, usually without drugs, has generally been the method of choice. Judd reports the results in a series of 100 consecutive operations done in 1914 and finds that 66 per cent show marked improvement and 5.5 per cent are slightly improved. Eleven of the 100 patients died after leaving the clinic. In a similar series studied in 1909 by the same author, only 44 per cent were

apparently cured. The difference in the two percentages is ascribed to improvements in technic.

The first attempts to treat thyrotoxicosis by the roentgen ray were made as early as 1905. Little has been done however, until during the last five years. Pfahler and Zulick believe that the roentgen ray should be used as a preliminary to surgical operation. They found that by this means the thymus was wholly or partially destroyed and risk from operation was reduced. Kocher had found the thymus enlarged in 50 per cent of his operative cases. The first symptoms of improvement under roentgen ray treatment were found to be increase in weight and decrease in pulse rate. The enlargement of the neck and the exophthalmos did not diminish until late and in many cases did not change.

Means and Aub conclude from their series at the Massachusetts General Hospital that as a rule rest in bed with x-ray treatment should first be followed and continued until the metabolism reaches a level. If this level is not within 20 per cent of the normal it is proper to resort to surgery unless there is a counter indication such as a rising metabolism in spite of complete rest. If the metabolism rises following operation, roentgen therapy should again be applied. Treatment should not be measured by weeks, but by months and often by years.

Hodges believes that early mild or questionably toxic goiters should receive roentgen treatment; that moderately severe cases apparently do better after surgery, and that the severe cases should be irradiated preliminary to the operation. When the result, following operation, is incomplete or unsatisfactory, the roentgen ray will frequently clear up the remaining toxicosis.

Holmes and Merrill feel that roentgen ray treatment accompanied by rest should be tried in all cases and should be continued long enough to destroy at least the thymus before resorting to surgery. They emphasize, however, the importance of a careful diagnosis as to the type of goiter. In simple or colloid goiters and in cystic goiters there is no evidence of hyperplasia of the gland and there are no toxic symptoms. Radiation would only destroy the remaining portion of the normal gland and would hasten hypothyroidism.

These investigators also emphasize the importance of continuing treatment over a long period. In their series, decided improvement was usually seen twelve months after the beginning of treatment. Usually at the end of six months the patients had sufficiently recovered to resume their ordinary occupations, and after one year there was a considerable diminution in the size of the thyroid gland. The results did not appear to be obtained as rapidly as after surgical intervention, but relief appears to have been more permanent, and the danger in the treatment is decidedly diminished.

The dangers of roentgen ray treatment consist in, first, the possible destruction of too great an amount of thyroid substance, with resulting hypothyroidism; second, the possibility of telangiectasis and atrophy in the regions treated. This is particularly to be avoided in young women in whom the resulting disfigurement gives considerable concern. Third, the toxemia of hyperthyroidism may be increased to a dangerous degree by the first

treatment. This may be guarded against by starting with small doses, preceded by rest in bed with medical treatment. Fourth, cases which have had preliminary surgical treatment and are then treated with the x-ray are particularly liable to hypothyroidism and require carefully controlled treatment.

Aiken has used radium in the treatment of toxic goiter and claims equally good results to those following the x-ray. This method also must be reinforced by proper medical care. It is important that the radium be screened so as to prevent the action of the short but powerful beta rays and to obtain the benefit of the more penetrating gamma rays.

The mode of action of the rays has not been clearly worked out. Waters in experimenting on dogs found that the roentgen ray in moderate doses resulted in a cloudy swelling of the thyroid cells. With larger doses coagulation necrosis was produced. It is possible that in a gland showing hyperplasia, these results are even more marked. Hodges suggests that in mild hyperthyroidism, before much if any, hyperplasia has occurred, an induced cloudy swelling may be all that is necessary for successful results.

The x-ray or radium ray will destroy the thymus gland in a short time. Hodges reports that many very large thymus glands under his observation were so reduced within six weeks time that no shadow or widening of the mediastinum could be shown with the fluoroscope.

The method of application according to Holmes and Merrill should be as follows. Treatment should be applied to both the thymus and the thyroid regions. Fairly hard rays should be used and treatment should not be repeated until after a lapse of three weeks. After a series of three treatments there should be an interval of three months, then a second series of three treatments should be given. If the symptoms have not sufficiently disappeared at the end of this period, a third series should be given making nine treatments in all. The patient will be under observation for about one and a half years. This is the technic in general use.

Kingery has recently made a preliminary report in which he suggests another method of therapy. His experience appears to have been chiefly with dermatologic conditions and not with toxic goiters, but the principle involved would appear to be similar in both cases. This author points out that in the older method of roentgen therapy maximum effects were gradually obtained by the frequent administration of small doses over a prolonged period. This is known as the method of "fractional dosage." The method of "massive doses" involves the administration of a maximum dose at one time followed by a prolonged interval without treatment. In either method there is an interval in which the tissue effects are not definitely known. In the first this phase precedes the stage of the erythema that results from cumulative effect. In the second method the uncertain period follows the original maximum effect.

We are accustomed to consider the roentgen ray as being stimulative in small doses, inhibitory in large doses and destructive in very large doses. Kingery points out that in the period of low or partial saturation, preceding or following maximum effect (erythema dose), there may occur a not inconsiderable amount of stimulation. This, of course, is not to be desired. He be-

lieves that the reaction between tissue cells and the radioactive substance follows a law similar to the law of mass action. The greater the concentration of the biochemical products of irradiation, the higher the velocity of their loss. As this concentration decreases, the velocity of loss becomes less in direct ratio. At such a time as this concentration is decreased by one half, the velocity of loss will have become less by a similar amount, and so on until the residual effect has become negligible. This rate of loss theoretically would represent a logarithmic curve.

If such be true, Kingery points out that the curve of residual effect in exposed tissues is logarithmic. The greater the amount of rays absorbed, the higher will be the initial velocity of loss.

From past experience it is known that the residual effect from an erythema dose has become negligible around the fourteenth day after treatment. With this as a basis Kingery constructs a logarithmic curve the ordinates of which express percentage of saturation of the tissues with rays, and the abscissæ the days elapsed since the single massive radiation. With this as a basis he calculates that the tissue saturation at the end of seven days is but 25 per cent while at the end of three and one-half days it is fifty per cent. He proposes now to treat at the end of three and a half days with half of an erythema dose, a procedure which would result in raising the saturation once again to 100 per cent. If treatment is given every three and a half days with 100 per cent saturation the first time and 50 per cent of a full dose on succeeding periods, the saturation of the tissues will at no time fall below 50 per cent. In this way he hopes to eliminate the uncertain period in which stimulation effect may occur. He has found it inadvisable up to the present to decrease intervals to less than half a week.

It is difficult to weigh the relative advantages of the roentgen ray over other methods, in the treatment of toxic goiter. As Holmes and Merrill have pointed out, Hale and White found that out of 87 cases treated medically, with only supportive therapy, 61 ultimately recovered, 21 were decidedly improved and only 5 remained unimproved. Stanton believes that exophthalmic goiter is a self-limiting disease and that 60 to 70 per cent of cases result in spontaneous recovery at the end of five or six years. These figures are very similar to the 66 per cent reported by Judd after surgical treatment.

Hodges found that in his series 50 per cent were apparently cured and 32 per cent greatly benefited by x-ray treatment.

Holmes and Merrill found decided improvement in most of their cases within 12 months after treatment was begun. They recognized that the symptoms had often been present for a considerable time and that possibly the disease had nearly run its course before treatment was begun. Nevertheless they believe that the evidence is sufficient to show that in early cases a more ready response to treatment and more satisfactory results are obtained by roentgen ray therapy.

From a study of these various observations, it would appear that the percentage of recovery by medical, surgical or roentgen ray treatment, individually, is about the same. It may be that following the latter methods recovery is swifter. In many cases without doubt a combination of all three

methods gives the best results. Treatment is most successful when it is individualized. Greater refinements in the technic of radiotherapy and new methods such as the application of Kingery's method to treatment of the thyroid gland may be productive of more satisfactory results. In the mean time it is well to recall that our present methods are but therapeutic patch work as long as we remain ignorant of the pathogenesis of the disease.

—*W. T. V.*

Late Sequelae of Encephalitis Lethargica

ALTHOUGH "lethargic encephalitis" as such has been described since 1916, attention has been devoted to it particularly during and since the 1918 influenza epidemic. Recently Durand has asserted that the disease is by no means new, that epidemics with quite similar features have been reported from time to time in various countries. A great difficulty in its identification is the large variety of clinical symptoms which may occur in different cases. Epidemiologic considerations aid greatly in the diagnosis. While according to Netter, lethargy and ocular palsies are present in as high as 75 per cent of cases, yet there are many in which the symptoms are complex and simulate other diseases of the central nervous system. This is to be expected with a disease which is apparently infectious and which may localize at various widely separated areas in the brain and spinal cord. Almost any combination of nervous symptoms may occur. Fever is usually present.

Dunn and Heabey have recently described eight clinical groups. It must be borne in mind that the individual members of these various classes all differ somewhat from one another and that the classification is at best artificial. There are cases simulating paralysis agitans and others resembling the condition known as disseminated sclerosis. In the individual case the general features are more valuable in making a diagnosis than is the determination of the portions of the central nervous system involved.

Remond and Lannelongue have followed for a period of ten months, five individuals convalescent from an attack of encephalitis lethargica. Four were men and one was a woman. Some of these had presented paralytic symptoms while others showed clonic manifestations with a remarkable diminution of psychic activity and a marked tendency to somnolence. Two reactions, however, were common to all cases, first a dissociation between the pupillary reaction to light and accommodation, and second an increase above the normal of sugar in the cerebrospinal fluid (.65 to .80 grams.) The pupillary reaction in accommodation was found to be definitely slower than that to light.

These authors were particularly interested in the extreme tenacity and the variety of the pathologic phenomena, and the frequency of relapses. The first patient still complained of symptoms three months after onset of illness; the second, seven months; the third, eight months; the fourth nine months. The fifth, nine months after onset, had been in the hospital for his illness, four times.

Among these five individuals the symptoms were as diverse as they are during the primary attack. Perhaps the most frequent symptoms in the cases were a persistence of the tendency to somnolence and a hyper-irritable condition of the peripheral nervous system. As the authors suggest, the disease is an affection of the nervous system which develops very rapidly and terminates either by death or by recovery, but which in certain cases, becomes prolonged into a chronic affection with symptoms essentially of prostration and hyper-irritability. This irritability sometimes affects the peripheral nerves, sometimes the posterior nerve roots, sometimes apparently the muscles themselves, and sometimes the special senses. Often the late cases resemble multiple sclerosis.

The increase of sugar in the cerebrospinal fluid may last as long as seven months. The authors believe that this indicates a persistence of the infectious agent.

Weber reports the case of a young man who fell a victim to an ambulatory attack of "lethargic encephalitis" in December, 1919. He was sick five months, during which time the predominant symptom was a somnolent condition. He was admitted to two hospitals subsequent to this, the second admission being in September, 1920. In this case the symptom complex resembled paralysis agitans. Janet has also reported an ambulatory case in a child of twelve years which was followed by symptoms resembling paralysis agitans. Similar observations have been made by Kinier Wilson, Economo and by Tretiakoff and Bremer.

It is well in the future to consider "lethargic encephalitis" as an important antecedent in the diagnosis of certain diseases of the central nervous system.

—*W. T. V.*

Mexican Smallpox

IN view of our recent editorial, "Are there two diseases included in the present diagnosis of Smallpox", we have received a copy of a most valuable article on smallpox written by Dr. Frank R. Young of Gering, Nebraska. This article was published in the December 1914 number of the Journal of the Arkansas Medical Society. Dr. Young reports in this article a highly fatal epidemic of smallpox which was imported from Mexico. He states:

"Mr. White and family left Tampico, Old Mexico, on December 30, 1913, landed at Port Arthur on the night of January 6, and reached Bert Carson's home in Elm Springs Township on January 8. On January 5, a boy, two and one-half years old, became sick, but his disease was not diagnosed as smallpox until January 10, before which time a number of people had been exposed to the disease. The epidemic spread, finally causing the sickness of twenty-six people. Of these ten died. Of those that died, but one had been vaccinated and he had been vaccinated in 1864. Of the vaccinated people who were in almost constant contact with the patients were Mr. and Mrs. White, two White children and Mr. and Mrs. Phillips. Of these, Mr. and Mrs. White and one White child did not contract the disease. One

White child had the disease in a very mild form, not necessitating going to bed. Mr. and Mrs. Phillips each had the disease in a very mild form. Mr. and Mrs. Phillips were vaccinated in 1864, and no attempt to revaccinate had been made since that time until after this outbreak. The White family were vaccinated in 1910. A number of other persons, who had been vaccinated, came in contact with the disease before its nature was known, and did not contract it. Alex Downum, who had a severe attack of smallpox in the year 1864, at the age of two years, was employed as a nurse in the Phillips home and contracted the disease in a moderately severe form. Other persons, numbering in all about twenty, who had had the disease previously, were employed as nurses and none of them contracted the disease."

This is a very interesting report and we are under obligations to Dr. Young for calling our attention to it. Smallpox of a malignant type was introduced into Mexico at the time of the Conquest by Cortez and his soldiers. This is the Asiatic form of smallpox. It was malignant at that time and has remained so ever since.

—*V. C. V.*

Fiftieth Annual Meeting of the American Public Health Association

THE fiftieth annual meeting of the American Public Health Association will be held at New York City in November, 1921. The date which is tentatively announced is November 14-18.

It is interesting to note that Dr. Stephen Smith, the founder and first president of the Association, is now entering his 99th year. He is still active and vigorous and is expected to celebrate his approaching centennial together with the semi-centennial of the Association.

The first organization meeting of the Association was held in New York City on April 18, 1872, and that is one of the reasons for selecting New York City for the celebration of the semi-centennial. Other considerations are the convenience to foreign representatives and to Dr. Smith, who lives in New York City; and especially a plan to conduct demonstrations of public health administrative methods in the laboratories, executive offices, garbage disposal plants, and similar centers of public health interest, in which New York City is unsurpassed.

It is expected to present in connection with the celebration a review of the progress of the various branches of public health within the last fifty years. The sectional programs will include Public Health Administration, Vital Statistics, Laboratory, Food and Drugs, Sociology, Sanitary Engineering, Industrial Hygiene, and Child Hygiene.